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HEREDITAS

GENETISKT ARKIV

UTGIVET AV MENDELSKA SÄLLSKAPET I LUND

REDAKTÖR: ROBERT LARSSON

BAND XXXVI HÄFT. 3

LUND 1950, BERLINGSKA BOKTRYCKERIET

HEREDITAS,

a periodical devoted to the publication of original research in genetics and cytology, is published by the MENDELIAN SOCIETY of Lund, Sweden. All contributions are written in English, German or French, illustrated, when necessary, with adequate text figures and plates. The current volume (XXXVI) will contain about 500 pages, issued in four numbers.

Subscriptions should be sent to the Editor. Price *Twenty-five* (25) *Swedish Kronor per volume* (postage free). All back volumes are still available. The price of Vols. I—X is 10 Swedish Kronor per volume, of Vols. XI—XXIII, 15 Sw. Kr., of Vols. XXIV—XXV, 20 Sw. Kr., and of Vols. XXVI—XXXV, 25 Sw. Kr. Thus, the price of a complete series of the periodical — Vols. 1—XXXVI (1920—1950) — is 610 Swedish Kronor (postage free).

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STUDIES ON THE CHROMOSOMES OF ULOPHYSEMA ÖRESUNDENSE

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I. INTRODUCTION.

SOME years ago BRATTSTRÖM (1936) described a crustacean belonging to a new genus of *Ascothoracica*, represented by the species *Ulophysema öresundense* BRATTSTRÖM living as a parasite in the coelom of sea-urchins, especially *Echinocardium cordatum* PENNANT. Since then BRATTSTRÖM (1947, 1948 a, b) has treated the species very thoroughly from various points of view. Thus, in most respects it is the very best known species of *Ascothoracica.*

No researches on the karyological conditions of the animal have been made. The present study reveals that the chromosomes of the organism and their behaviour are very remarkable.

II. BIOLOGICAL NOTES.

It is necessary to give some information on the biology of this highly specialized animal in order to make the following description of the chromosomes intelligible.

According to BRATTSTRÖM, the parasitic females of *Ulophysema öresundense* live as adult animals in the coelom of sea-urchins, where specimens measuring from 0,5 to 22 mm. may be found. Small specimens lie at different places in the host, but when they have reached the size of about 7—8 mm. they have generally moved to the inside of the sea-urchin's test, where they are able to corrode a small hole and thus establish connection with the external water.

The most prominent part of the animal consists of the enlarged so-called mantle, which forms a walnut-like wrinkled vesicle. Ontogenetically the mantle consists of folds from the lateral parts of the nauplian body.

Several hundreds of eggs are produced periodically from the ovaries of *Ulophysema öresundense.* They fall almost simultaneously into the mantle-cavity, which serves as a brood-chamber. The eggs here develop into cypris larvae, which are able to escape through the above-mentioned

opening in the test of the host and to swim into the free water masses. The difficulty of accounting for fertilization suggests that parthenogenesis is a possible mode of reproduction.

In 1948 BRATTSTRÖM (1948 b) described cypris larvae with testes and spermatozoa. It is probable that these males have some sexual function, and, if parthenogenesis occurs, it is not the only mode of development in the species. BRATTSTRÖM believes that all cypris larvae are males and that later on they will infect sea-urchins and develop into female parasites. This, however, is probably not the case, since only half of the larvae have testes. The sex ratio is indicated in Table 1.

TABLE 1. *The sex ratio of Ulophysema öresundense.*

The table shows the distribution of 1100 metanauplius and cypris larvae with testes (♂♂) and without gonads (♀♀). The samples have been taken at random from the mantle-cavity of seven parasitic female specimens.

Number of ♂♂	Number of ♀♀
45	55
160	140
53	47
49	51
110	90
80	120
48	52
Total: 545	555

It will be seen that a distribution of 1 : 1 among the two sexes is almost certain. As a consequence the sex-determination is probably brought about by means of sex-chromosomes or sex-genes. My observations indicate that there are probably sex-chromosomes in this case.

III. MATERIAL AND METHODS.

Some specimens were collected by the present author from the Sound between Sweden and Denmark in the summer of 1948. All sufficiently large specimens of *Echinocardium cordatum* were dissected immediately after the dredging. The *Ulophysema* specimens which were found in them were fixed and permanent squash-preparations for chromosome studies were made later according to MELANDER (1948). The stain was aceto-carmine or orceine. Some specimens which were fixed in the same way by BRATTSTRÖM have also been available. In addition I have examined sections earlier used by BRATTSTRÖM, and I have made

other sections of material which he had fixed in BOUIN or 80 % alcohol. The stain was here HEIDENHAIN's haematoxyline.

I owe many thanks to Professor Dr. HANS BRATTSTRÖM, who permitted me to use his material of *Ulophysema.*

IV. KARYOLOGY.

The karyological conditions of *Ulophysema öresundense* were studied to a large extent on squash-preparations from the mantle-wall. Large parts of the proximal mantle are occupied by strings of vitellarian cells following the branched gut-diverticles (MELANDER, 1950). When the vitellarium of an animal is growing, the border parts of its strings show a violent mitotic activity. Most vitellarian cells remain in the prophase stage until they are used in the yolk-formation, but the cells of the gut-diverticles and the mesenchyme and also the epithelial cells have most nuclei in the interphase-stage. The nuclei of the epithelial cells belong to a special size-category with a diameter that is only about one-third of that of the others.

According to my interpretation (see below), the majority of all mantle cells (also those of the epithelium) are tetraploid or nearly tetraploid.

Thus, when part of the proximal mantle-wall is being crushed for the purpose of chromosome studies, two size-classes of nuclei both having the tetraploid number (about 36) of chromosomes (with certain exceptions) are mainly found. Among the large nuclei there are many divisional stages, especially in animals with small growing eggs.

V. MITOSIS.

1. THE INTERPHASE.

Since the chromosome conditions of *Ulophysema öresundense* deviate from those of most other organisms, I will here define what I call a chromosome, and illustrate by a figure how it is principally built up in the late *mitotic* prophase.

Another possible interpretation would be to call each of the so-called chromatids (Fig. 3) a chromosome. This, however, would not alter any facts but only the terminology.

The conditions during the interphase are illustrated by Fig. 1.

There are three noticeable regions which are stained in different degrees (*h*, *n* and *c* in the figure; *h* is divided into two parts in this case).

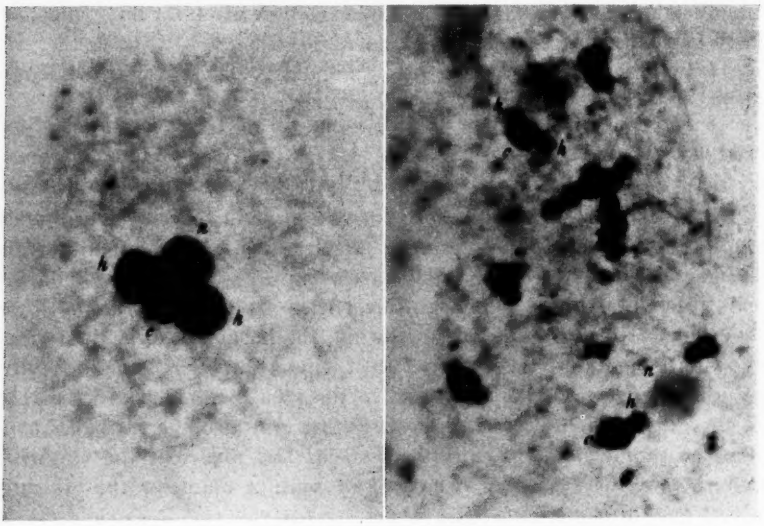

Fig. 1. Fig. 2.

Fig. 1. Mitotic interphase, c = collasome, n = nucleolus, h = positively heteropycnotic chromosome-ends attached to the collasome. In this case there are two such bundles (h). — × 1400. — Fig. 2. Mitotic interphase nucleus. (c, h, n as in Fig. 1.) In this case the collasome is divided into several separate parts. — × 1400.

Heteropycnosis. — Positive heteropycnosis during the interphase in the distal chromosome-ends (distal end = the end opposite the proximal one provided with kinetochore) is a common feature in all the chromosomes of this species. One kind of stained regions in the interphase nucleus is composed of these chromosome-ends (h). The heteropycnotic parts of the chromosomes lie together alongside each other, which indicates that they are sticky.

The nucleolus. — The most faintly stained region in the interphase nucleus (n) is the nucleolus, which seems to be organized by only one of the members of the haploid complement. According to the mode of arrangement, the nucleolar chromosomes in a polyploid nucleus organize either one or several separate nucleoli. If two or more nucleolar chromosomes lie together, there is apparently only one nucleolus to be seen, which in reality is composed of several nucleoli sticking together.

The collasome. — The darkest of the stained regions (c) lies in the

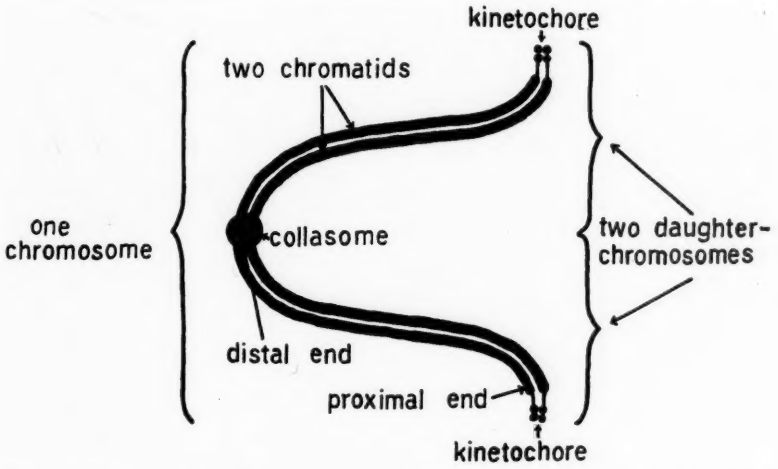

Fig. 3. Scheme of a mitotic chromosome of *Ulophysema öresundense* as interpreted by the author.

centre of Fig. 1 close to the nucleolus. It is difficult to analogize it with any known cell-organ, and in order to facilitate the description of the chromosome conditions of *Ulophysema öresundense* I think it expedient to denominate it and I propose the term *collasome* (Greek: *colla* = glue and *soma* = body). It has the property of sticking the positively heteropycnotic distal chromosome-ends to its surface. In this way the chromosomes become arranged into bundles (constituting the regions *h*) with their distal ends at the surface of the collasome. All the chromosomes of an interphase nucleus do not necessarily form only one aggregate (including the regions *h*, *n*, *c*) as in Fig. 1, but generally the collasome is divided into several parts and consequently the chromosomes are arranged in several separate aggregates (Fig. 2).

It seems as though the collasome is not composed of real chromosome parts. Probably, it is organized by chromosomes in a similar way as is the nucleolus, but each and every chromosome of the complement and not merely one of them is able to organize it. The collasome-organizer is situated at the distal chromosome-end (or close to it). Like the nucleolus the collasome is largest during interphase, when it is also most deeply stained.

The function of the collasome during the divisional cycle of the nucleus will be further discussed below.

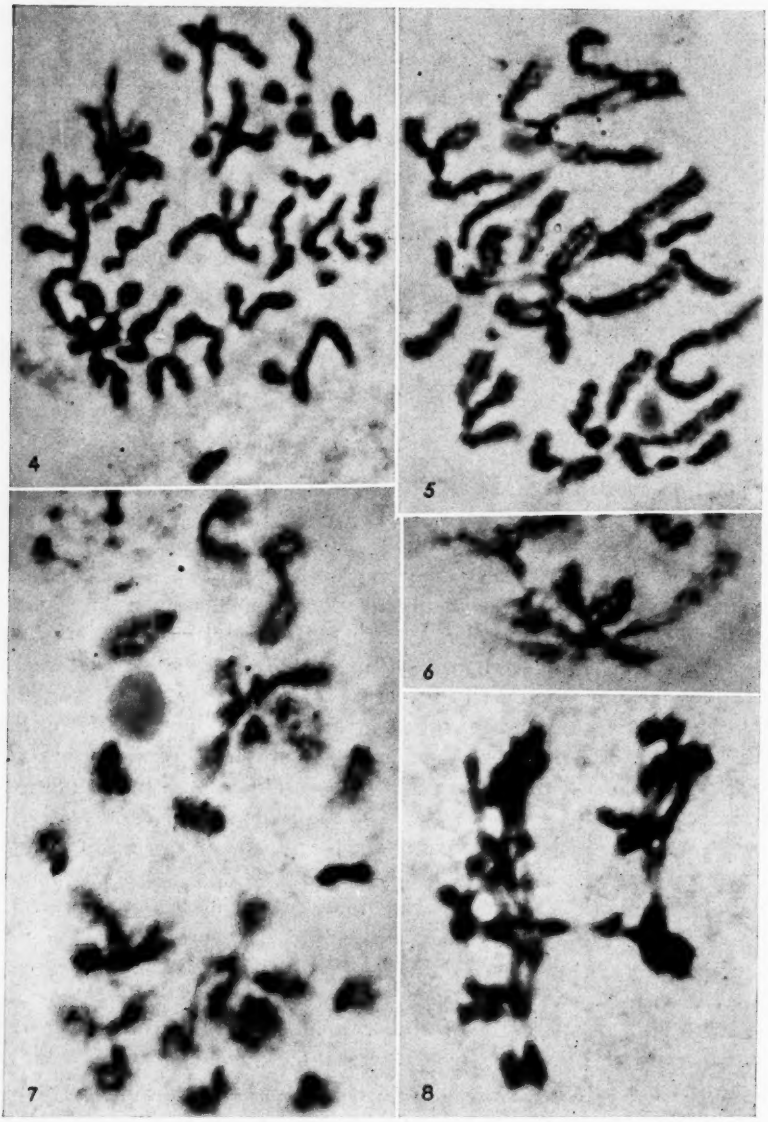

Fig. 4. Middle mitotic prophase. 8-ploid nucleus from the mantle-wall. To the right in the upper part of the figure a nucleolus and its organizer are visible. — × 1400. — Fig. 5. Middle mitotic prophase. 4-ploid nucleus from the mantle-wall. Note remaining

2. THE DIVIDING NUCLEUS.

The prophase. — At early prophase the nearly unstained proximal chromosome parts begin to be stainable. Now each chromosome has two bundles of chromatids, each composed of at least two threads (Figs. 4 and 5). The chromosome to the right in Fig. 6 shows at least 4 chromatids. At mitosis the chromatid division — as far as it can be studied under the microscope — seems to take place during interphase or possibly already between metaphase and the following early prophase. During prophase the chromosomes shorten and become more and more stainable (Figs. 7 and 8).

The nucleoli decrease in size. They are sometimes connected with chromosome-ends (Figs. 4 and 7) but often they lie free. The collasomes, too, decrease in size during prophase and they do not take up so much dye as before. At later prophase only slightly stained thin strings, which are remains of collasome, still keep the chromosomes together in bundles (Figs. 5, 7 and 8).

The kinetochores. — At late prophase the kinetochores become visible (Fig. 9). There are two in each chromosome, situated opposite the collasome-end. Thus, even before prophase the chromosomes are divided into two future daughter-chromosomes, which are connected only by the aid of the collasome. Each chromatid — there are two visible in each daughter-chromosome — ends in two minute and clearly stainable globules, which are separated by less stained material. Thus, the functioning kinetochore consists of four stained components lying close together in pairs (Fig. 3). However, usually only one globule can be seen under the microscope, but nevertheless some preparations of late metaphase- and of anaphase-chromosomes show the compound nature of the kinetochore. Thus, its division takes place before late prophase.

Before the spindle is formed the kinetochores point in the direction of the prolongations of the other parts of the chromosomes (Fig. 9). At late prophase, however, the kinetochores of two daughter-chromosomes

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parts of the collasome connecting the distal ends of the chromosomes. At the top of the figure a nucleolus and its organizer are visible. — × 1400. — Fig. 6. Middle mitotic prophase. To the right a chromosome consisting of at least 4 chromatids. — × 1400. — Fig. 7. Middle mitotic prophase. About 4-ploid nucleus from the mantle-wall. Note remaining parts of the collasome connecting the distal chromosome-ends (especially in the lower part of the figure in the middle). — × 1400. — Fig. 8. Late mitotic prophase from the mantle. All the chromosomes are connected by remaining parts of the collasome. This is very often the case. — × 1400.

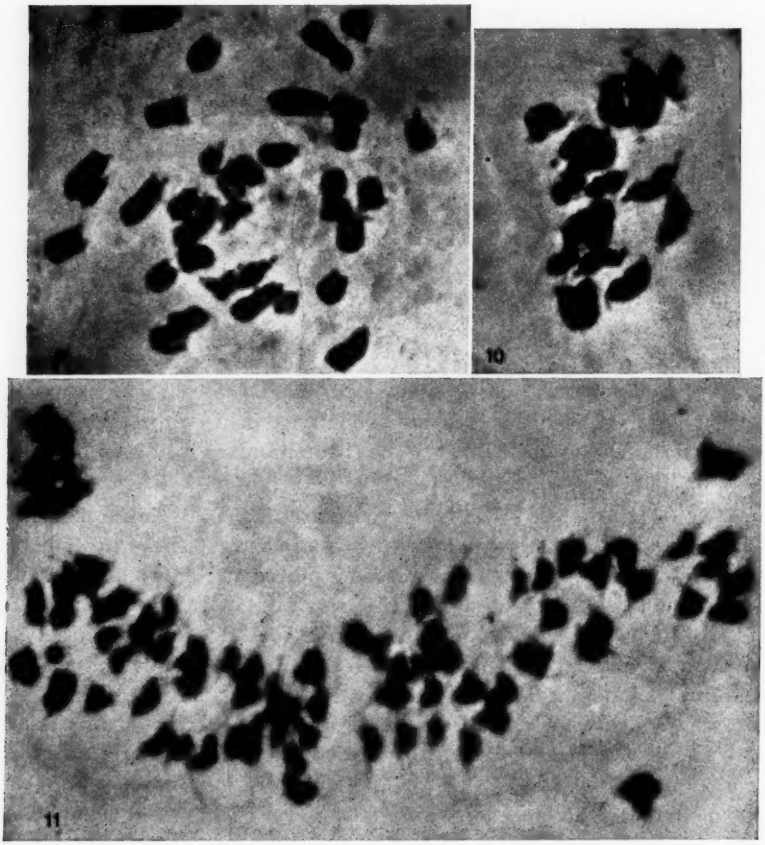

Fig. 9. Late mitotic prophase. About 4-ploid nucleus from the mantle-wall. The kinetochores are visible, two of them at each chromosome-end. The kinetochores point in the direction of the rest of the chromosome. — × 1400. — Fig. 10. Early mitotic metaphase. Diploid nucleus from the mantle-wall. The kinetochores form an angle with the rest of the chromosome. — × 1400. — Fig. 11. Mitotic metaphase. About 8-ploid nucleus from the mantle-wall. The kinetochores have started their movement to the poles. Note an exceptional, small chromosome to the left in the figure. — × 1400.

begin to point towards opposite poles and to form an angle with the rest of the chromosome (Figs. 10 and 11).

The metaphase. — It is of course most expedient to call the stage at which the chromosomes are orientated in a plate and after which

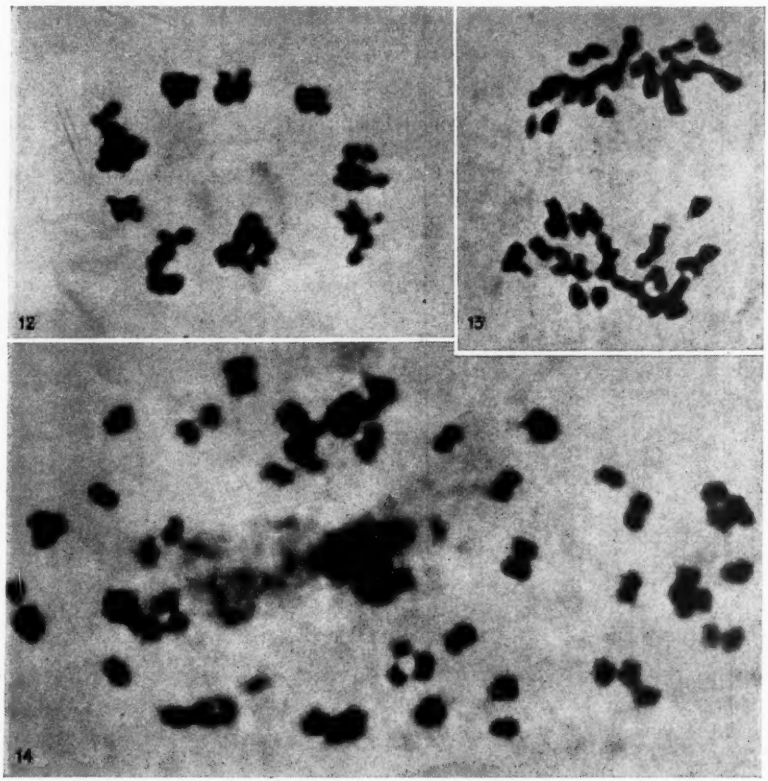

Fig. 12. Late mitotic metaphase following an endomitotic chromosome doubling. The chromosomes still lying in pairs. From the mantle-wall. — × 1400. — Fig. 13. Mitotic anaphase. From the mantle-wall. Note that no kinetochores are visible at the chromosome-ends. — × 1400. — Fig. 14. Endomitosis within the nuclear membrane in a cell from the mantle-wall. The earlier divided chromosomes lying in pairs. — × 1400.

a separation of daughter-chromosomes follows metaphase, although in the present case the kinetochores are already divided, contrary to what is common among other organisms. It is strange to imagine *mitotic* metaphase chromosomes each provided with two different kinetochores. At the first glance one gets the impression that meiotic bivalents (Figs. 10 and 11) are involved.

In this connection reference is made to the mitotic chromosomes of *Uvularia* studied by BARBER (1941) and the accessory chromosomes

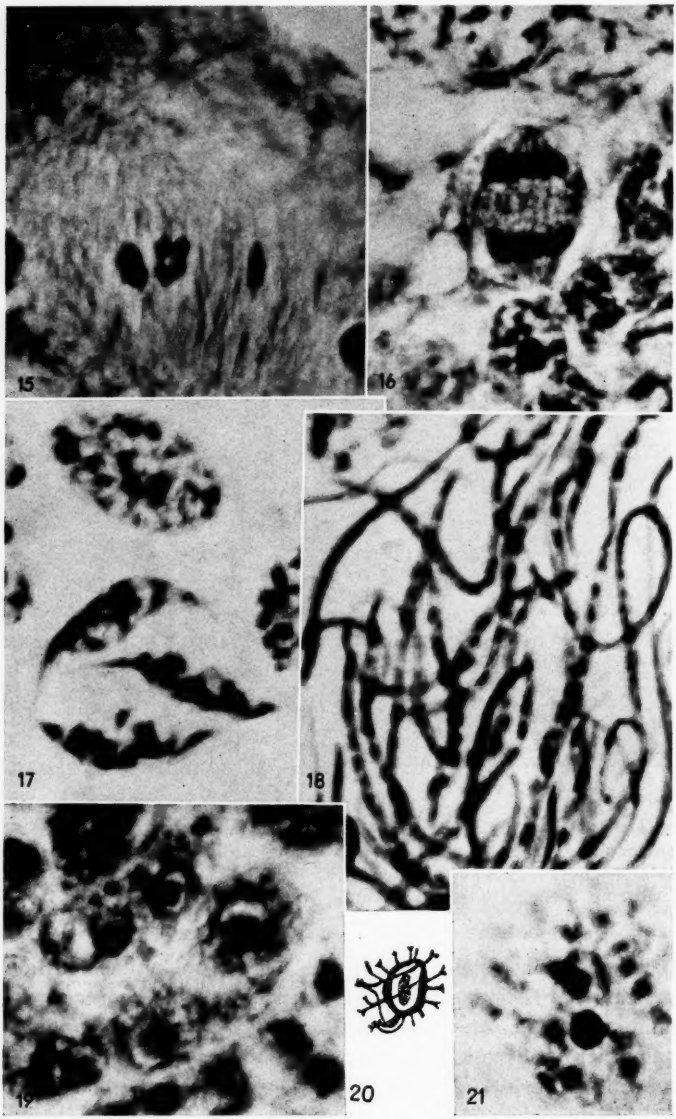

Fig. 15. Late metaphase of the first mitosis of the zygote. Section comprising part of the nucleus. — × 1400. — Fig. 16. Mitotic anaphase from an embryo. The kinetochores at the poles connected with the chromosomes by the thin kinetochore-necks.

of rye studied by MÜNTZING (1946). In both cases the centromeres are divided before the separation of the daughter-chromosomes takes place. These mitotic chromosomes also resemble bivalents to a certain degree.

When the full metaphase is reached in *Ulophysema* all the chromosomes are orientated in one plane forming a circle (Fig. 12). Thus, a »hollow spindle» is typical.

The daughter-chromosomes are gradually pulled towards the poles but they still stick together at their distal ends where the sticky remains of the collasome are situated (Fig. 15). If several chromosomes are connected into bundles during interphase and early prophase, this connection will remain at metaphase as well. This explains the common occurrence of clusters of chromosomes in the metaphase plate.

The force acting on the kinetochore causes the proximal chromosome-end to point towards the pole, and finally the two daughter-chromosomes form one straight line between the stretched kinetochores (Fig. 15). As said above, they were previously lying together with only the kinetochores pointing towards the poles (Figs. 10 and 11). Ultimately, however, the stickiness disappears and the daughter-chromosomes start their movement towards the poles (Figs. 13 and 16).

The anaphase. — The globules of the kinetochore start their movement towards the pole before the rest of the daughter-chromosome. The globules are then connected with the latter by very thin threads. At anaphase these threads seem to form a minute spiral, which pulls the daughter-chromosomes towards the pole (Fig. 16). In the present fixations it is sometimes possible to see the globules of the kinetochores on their way to the poles at late metaphase. Accordingly, at full anaphase no globules can be seen situated close to the chromosome-ends (Fig. 13) but only what seems to be aggregations of them near the poles. This interesting feature, however, cannot be cleared up at present owing to lack of homogeneous fixations. I hope to be able to study this problem later in more detail.

— × 1400. — Fig. 17. Three vestigial second meiotic »metaphases» within the spermatids. The small chromosomes which can be seen in them are similar in form to mitotic metaphase-chromosomes. No spindle is developed. — × 1400. — Fig. 18. The small chromosomes within the elongated spermatids are visible. — × 1400. — Fig. 19. Section of the testes from a male metanauplius larva. Some collasome-ring stages between the first and the second meiotic metaphases are visible. See also Fig. 20. — × 1400. — Fig. 20. A drawing of a nucleus in the collasome-ring stage between the first and the second meiotic metaphases in the male. In the middle the nucleolus. The ring is the collasome to which 18 double chromatids are attached. See also Fig. 19. — × 1400. — Fig. 21. A mitotic telophase nucleus from the mantle-wall. The dark collasome is organized by the chromosome-ends. — × 1400.

The telophase. — At early telophase the collasomes are reorganized at a time when the chromosomes still lie close together near the poles. In this way all or most of them become attached to one or sometimes several separate collasomes before they are despiralized in any essential degree. A telophase-nucleus has the appearance shown in Fig. 21.

The reader is referred to the scheme (Fig. 26) in the Discussion, which illustrates the conditions during mitosis.

3. ENDOMITOSIS.

Nearly all the cells from the mantle of adult female specimens are more or less tetraploid, but there are a few diploid nuclei, too, and a few which are octoploid. Very young embryos of both sexes have more or less the diploid chromosome number in most of their cells. Very soon during the development, however, tetraploid cells occur. They become more and more predominant in the somatic tissues as the development of the embryo goes on.

The endomitotic regulation of the chromosome number is a chromosome division without division of the nucleus in the sense of GEITLER (1938). In *Ulophysema* the chromosomes contract and become visible within the nuclear membrane during endomitosis (Fig. 14). Presumably, there is a despiralization and a disappearance of the visible chromosomes after this extra contraction, but I am not quite sure about it. No spindle at all develops during endomitosis.

As a result of the previous extra chromosome division the contracting chromosomes within the nuclear membrane lie in pairs (Fig. 14). After the endomitotic doubling within the nuclear membrane the chromosomes of the following normal metaphase still lie in pairs (Fig. 12).

VI. MEIOSIS.

1. THE SPERMATOGENESIS.

BRATTSTRÖM (1948 a) described the metanauplius- and cypris-larvae, but he did not analyse the spermatogenesis. Since the available material did not allow a very thorough analysis, the present description of the spermatogenesis of *Ulohpysema öresundense* should be considered only a preliminary one. I am well aware that misinterpretations are possible.

A. THE FIRST MEIOTIC DIVISION.

The prophase. — I do not know exactly what happens in the prophase nuclei of the spermatocytes. More suitable fixations are necessary

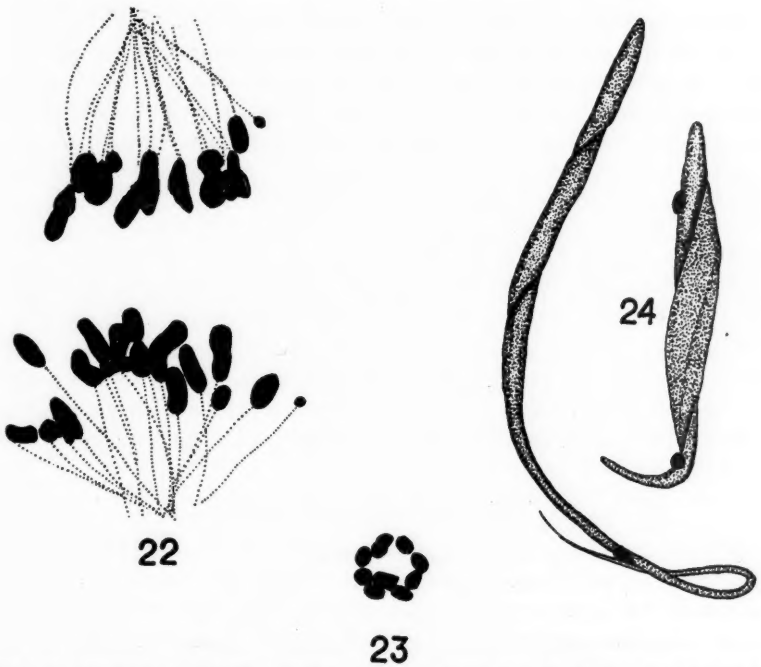

Fig. 22. Drawing of a diploid mitotic anaphase from a male larva. Note the small chromosome to the right in the figure, which is presumably the *Y*. — × 1900. — Fig. 23. The 9 chromosome configurations at the first meiotic metaphase of the male. — × 1900. — Fig. 24. Two spermatozoa. The dark globulae are the collasomes. — × 1900.

before this can be cleared up. What I think happens is illustrated by the scheme (Fig. 26) in the Discussion.

The metaphase. — There are nine chromosome configurations at metaphase of the first meiotic division or sometimes even more (Fig. 23). In some cases I saw that one of them was somewhat negatively heteropycnotic and divided a little before the others. Probably this is composed of the sex-chromosomes. One small part may be the *Y* and one large part the *X* judging from a comparison of the mitotic chromosomes from males and females (note the small chromosome in Fig. 22 of a ♂ which cannot be found in the other mitotic figures, which are of ♀♀). The sex-ratio, which is 1:1 (Table 1), is evidently in accordance with an *XY* sex-determining mechanism.

The anaphase and the telophase. — At anaphase usually 9 chro-

mosome-bodies are carried to each pole. At early telophase the collasome and the nucleolus are reorganized. The collasome assumes the form of a circle with 18 double chromosome-threads attached to it by their distal ends. The nucleolus lies in the middle of the collasome-circle. These odd nuclei can be seen in Figs. 19 and 20. Sometimes the collasome-circle is defective and has the form of a horse-shoe. If so, the spermatozoa which develop out of these nuclei will be defective, too.

The interphase. — If there is a true resting stage between the first and second meiotic divisions, this interphase cannot be long, because generally the chromosomes are not quite despiralized during this stage. The cell with the nucleus containing the above-mentioned collasome-ring with its attached chromosomes begins to assume an elongated form. The stretching of the cell starts in one direction, but the lengthened part of the cell soon divides into two different protuberances pointing in opposite directions. Possibly this is connected with the centrosome and its division.

B. THE SECOND MEIOTIC DIVISION.

The chromosomes which are attached to the elongated collasome-ring contract to a form resembling that of mitotic metaphase chromosomes of the epithelium (Fig. 17). At this stage the collasome-ring is not so well developed and it is very little stained. There is no longer any nucleolus. All these features are commonly attributed to dividing nuclei. The only essential difference is the lack of a spindle and consequently the lack of a real division of the nucleus.

In the Discussion (Fig. 26) I have submitted what I believe happens during meiosis. In several respects this interpretation is hypothetic.

From the above description it is evident that the development of the independent spermatozoa (the spermatids) begins immediately after the first meiotic division. Consequently, there is no real second meiosis in *Ulophysema*. However, *the fact that the chromosomes become visible within the spermatids might be considered a rudiment of the second meiosis* in other organisms.

C. THE SPERMATOZOA.

After the »metaphase» (the stage with most contracted chromosomes) of the vestigial second meiosis the spermatid is more and more prolonged (Fig. 18). Then the chromosomes gradually come to resemble interphase chromosomes; they are despiralized once more and no longer visible under the microscope. The collasome is reorganized and each

of the fully developed spermatozoa contains one or more such bodies. As usual, they are easily stainable, and in this case they are situated at the surface of the spermatozoa, where they generally project like semiglobules (Fig. 24).

2. THE OOGENESIS.

In a previous paper (MELANDER, 1950) the present author has treated some features in the growth of the eggs of *Ulophysema öresundense*. It was pointed out there that meiosis is probably of short duration and goes on simultaneously in the cells of the ovaries of the female parasite. I have hitherto searched in vain for the proper stages.

VII. DISCUSSION.

In several respects a *mitotic* chromosome of *Ulophysema öresundense* behaves like a *meiotic* bivalent of many other organisms. For instance, there are two kinetochores in both, and a remaining part of the collasome in *Ulophysema* connects the two daughter-chromosomes at the mitotic metaphase just as chiasmata join the partner-chromosomes at the meiotic metaphase of most organisms.

At the mitotic metaphase several chromosomes are often connected with remaining collasomal parts. Thus, at this stage there are often clusters containing a number of chromosomes with twice as many kinetochores. Evidently, the orientation of the two kinetochores of a chromosome towards opposite poles is made more difficult when several chromosomes are connected in this way. This is shown by the fact that in some cases I have found that more kinetochores point to one of the poles than to the opposite one. The result must be an abnormal distribution of the daughter-chromosomes.

ÖSTERGREN (1945) was the first to show that the trivalents of a triploid, *viz.* in *Anthoxanthum aristatum*, do not lie in the equatorial plate but are dislocated in the direction of that pole towards which two of the centromeres point. The above-mentioned clusters of mitotic chromosomes of *Ulophysema* are also dislocated according to the same principle.

In some cases I have seen that an abnormal orientation of the kinetochores of a mitotic chromosome may occur even if the particular chromosome is not connected with other chromosomes as described above. Thus, sometimes the two kinetochores of a chromosome which is situated at the equatorial plate point towards the same pole. As a result

of this phenomenon there occur mitotic metaphase nuclei with most chromosomes in the equatorial plate but with one of them situated close to a pole. In this case both daughter-chromosomes are carried to the same pole. Obviously, this abnormal orientation is closely related to the well-known cases of failure of co-orientation which occasionally occur in ordinary bivalents at meiosis.

The exceptional orientation of the kinetochores towards the same pole, which is not so very rare, reveals that the chromosomes of *Ulophysema öresundense* are not quite adapted to the divisional mechanism in the animal, which may be considered an experiment in chromosome-mechanism less suitable than the normal mitotic process. The irregularities of the mitosis are reflected in the number of chromosomes found in somatic cells. It is by no means constant. The so-called primary vitellarian cells (MELANDER, 1950) are tetraploid or nearly so, the true tetraploid number being 36. The distribution of different chromosome numbers found in these cells is shown in Table 2.

TABLE 2. *The distribution of different chromosome numbers in the primary vitellarian cells of Ulophysema öresundense.*

Number of chromosomes	28	29	30	31	32	33	34	35	36	37
Number of plates:	2	0	5	0	10	2	31	1	34	4

The nature of the mechanism orientating the kinetochores of *Ulophysema* seems to be in accordance with the hypothesis of ÖSTERGREN to explain the co-orientation (manuscript in preparation). I assume on the basis of this idea that in *Ulophysema* the kinetochores attach themselves only at their terminal ends. That kinetochore which first happens to be orientated towards a pole will as a consequence of this be the first one to be pulled towards it. The resulting movement will immediately be transmitted through the weak kinetochore-neck to the second kinetochore via the two daughter-chromosomes — which are connected by the remaining part of the collasome. The second kinetochore will drag behind in this movement due to the resistance of the nuclear sap and to its attachment to the fibre structure of the spindle. As a consequence of this effect its terminal end will be orientated towards the opposite pole and pulled towards it. Finally, a balance will be established when the pulling forces are in equilibrium and the chromosomes lie in the equatorial plate.

The flexibility of the kinetochore-neck may be an important feature. This is illustrated by the model experiment shown in Fig. 25.

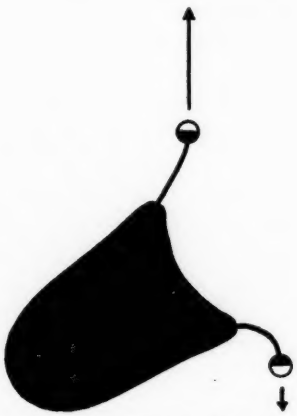

Fig. 25. Scheme of the co-orientation of the kinetochores. The white terminal part of the upper kinetochore is assumed to be pulled towards the pole indicated by the large arrow. Consequently the chromosome is pulled in the same direction and the lower kinetochore orientated with its terminal end towards the opposite pole because the kinetochore-neck is flexible and there is resistance within the spindle indicated by the small arrow.

If the kinetochore-neck were not sufficiently flexible, the force working in the above mentioned movements might not be able to produce the necessary bending of this chromosome region and both the kinetochores would be orientated and pulled towards the same pole. As said above, this is what may happen and it shows that the morphologic adaptation of the kinetochore is not absolutely reliable.

There seems to be no doubt that the mitotic anaphase chromosomes of several organisms are composed of more than one chromatid. Thus the chromosomes of coccids described by HUGHES-SCHRADER (1948) and those of *Allium* described by LEVAN (1949) show the compound nature of chromatids acting as units. My own work on the flatworms (unpublished) also shows that the anaphase chromosomes are double. Thus, it is not surprising to find at least two chromatids in some daughter-chromosomes of *Ulophysema*.

As said above, the nuclei of the epithelium are minute in comparison with those of most other cells, although they are tetraploid. The chromosomes are minute, too. The chromosomes which are visible in the spermatids during their development are also small and have a size comparable to that of the metaphase chromosomes of the epithelial nuclei (Fig. 17).

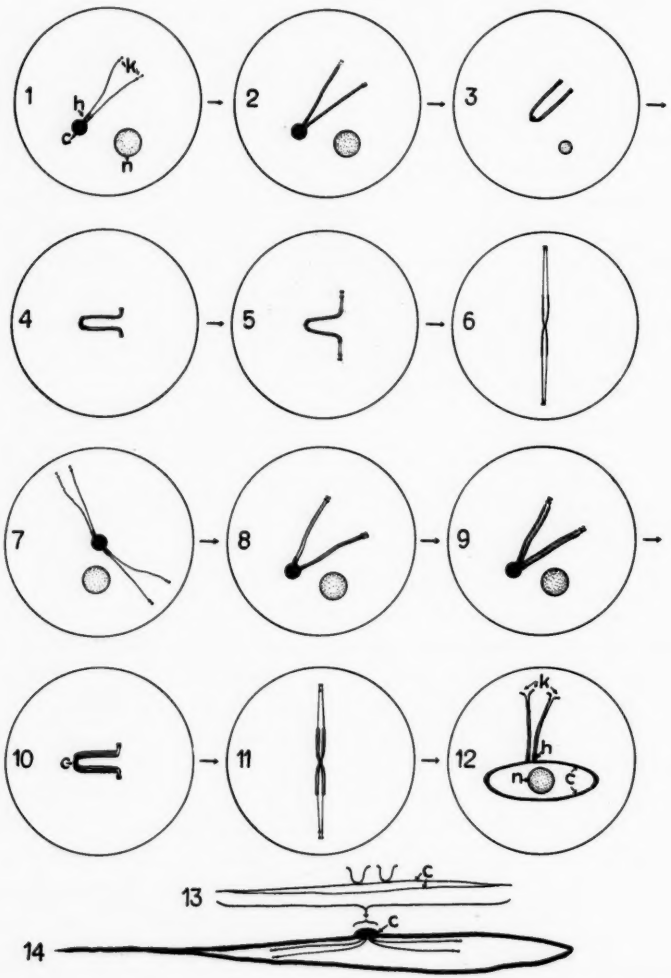

Fig. 26. Scheme of mitosis and meiosis in *Ulophysema öresundense*. *1—6*: mitosis: *7—14*: male meiosis (partly hypothetic). c = collasome, n = nucleolus, h = positively heteropycnotic chromosome ends, k = kinetochore. — *1*. Early prophase. *One chromosome* with *two chromatids* is figured. — *2*. The chromatids and the kinetochores divide. — *3*. Late prophase. The collasome and the nucleolus get smaller. The kinetochores point in the direction of the rest of the chromosome. — *4*. Early metaphase. The kinetochores point towards the poles and the chromosome is orientated in a plate. — *5*. The kinetochores move to the poles. — *6*. Late metaphase. The kinetochores at the poles are connected with the daughter-chromosomes which still stick

According to observations, these small chromosomes seem to be composed only of one single chromatid in each daughter-chromosome and not of double ones as in the big daughter-chromosomes of, *e. g.*, the primary vitellarian cells. This could explain the size-classes of chromosomes which actually exist, because one single thread spiralizes more easily into a smaller metaphase chromosome than two, provided they have the same thickness. The chromosomes might start with only one single chromatid (see below). According to this interpretation, most somatic cells are not only polyploid but their chromosomes are polytene as well.

There are a couple of facts which seem to be contradictory. Thus, there are two sexes in the ratio 1 : 1 and correspondingly the male has sex-chromosomes of the *XY* type. However, the chromosome number in the spermatozoa could be clearly shown to be 18, which is twice the number of chromosome configurations at the first meiotic metaphase. The oogenesis is not known, but in the very first mitotic division of the zygote there are distinctly 36 small chromosomes. Therefore the result of the female meiosis should be the same as that of the male meiosis, which means that the egg, too, contains 18 small chromosomes. In addition, there are two clearly different size-classes of chromosomes and the chromosome number is often only 18 in the nuclei of young embryos. At the early developmental stages both the size and the number of chromosomes are very variable.

If the following assumptions are made, all this can be explained: In principle the mitosis and the meiosis proceed according to the schemes in Fig. 26. The result of the meiosis may be $2(A + X)$ or $2(A + Y)$, ($A = 8$ autosomes) in the male and $2(A + X)$ in the female. All these chromosomes are minute ones consisting of only one chromatid in each daughter-chromosome [Fig. 26 (*13*)]. If there is no division of

together by some remnant collasome. — *7*. Early prophase of the first meiotic division. *Two chromosomes* with *four chromatids* are figured. — *8*. Pairing of homologous chromatids. — *9*. Splitting of chromatids. The kinetochores undivided or still attached to each other. — *10*. Orientation of the chromosome configuration at the first meiotic metaphase. Note that the kinetochores are still connected. — *11*. Late metaphase. — *12*. The collasome-ring stage. The chromatids are connected with the collasome-ring. The nucleolus is developed lying in the middle. The kinetochores are divided. — *13*. The »metaphase» of the vestigial second meiosis. There is no nucleolus and only a small residue of the collasome. The chromatids in the chromosomes are single and the chromosomes therefore minute. The result of the meiosis is a reduction of the number of chromatids but not of chromosomes. (The contour of the spermatid is not drawn.) — *14*. A spermatozoon which has an interphase nucleus with the collasome well developed. (The contour of the spermatozoon is drawn.)

the chromatids before the first mitotic division of the zygote, there will be 36 small chromosomes at this metaphase, as I have observed (Fig. 15 is from a section through part of the zygote). The zygote may contain: $4A + 4X = ♀$ or $4A + 2X + 2Y = ♂$.

If, under certain conditions occurring in some interphase nucleus close to the very first mitosis of the embryo, the homologous single chromatids of the small chromosomes [Fig. 26 (*13*)] pair two by two (or remain closely connected after their division at interphase), there will be 18 large chromosomes [Fig. 26 (*5*)] instead of 36 small ones because the collasome will connect these chromatid-pairs at their distal end and thus form new larger chromosomes. 18 large chromosomes occur only if all the chromatids are able to pair and to attach another pair at their distal ends. If this is not possible, there will be chromosome numbers between 18 and 36, and, in addition, the chromosomes will vary in size. This is exactly what I found.

There can never be any somatic chromosome pairing in the cells which form the epithelium because they always have minute chromosomes.

Pairing is facilitated because the collasome collects most or all the distal chromatid-ends during interphase. It is evident that the collasome influences the chromosomes very much, and ultimately most abnormal features are probably adaptations which occurred after its appearance.

LEVAN (1944) described the chromosomes of *Dipcadi*, which are nearly telocentric and stick together by means of the nucleolus during telophase and interphase. He regards this as the cause of the regular mitotic orientation of the chromosomes of the species. The present author has observed a glutinous substance connecting the chromosome-arms of a species of flatworms with iso-chromosomes (unpublished). The so-called chromocentre of certain *Diptera* may also be mentioned in this connection.

In these cases the chromosomes stick together only at telophase or also during interphase but no longer. The importanc of the stickiness is correlated to the orientation of the chromosomes and has nothing to do with the divisional mechanism as in *Ulophysema*. Here, the stickiness of the collasome is utilized mainly in two ways. Firstly, the existence of two different kinetochores during the time these are being attracted by the poles demands something to keep the two daughter-chromosomes together just as chiasmata do partner-chromosomes at the meiotic metaphase. Secondly, the collasome is useful for the orientation of the chromosomes at meiosis and during development of the spermatids and

the spermatozoa. It also makes possible the transition from small to large chromosomes.

The many abnormal features in the karyology of *Ulophysema* here described make this animal even more interesting than before. I hope that this paper, amplified by future studies and if possible extended to other species and related groups, will give some additional information of the disputed systematic position of *Ascothoracica* and also of the laws which govern the behaviour of the chromosomes.

SUMMARY.

The chromosomes of a parasitic crustacean, the ascothoracid *Ulophysema öresundense* BRATTSTRÖM, are described. (See Figs. 3 and 26.)

In the interphase nucleus each chromosome is composed of two chromatids, each of which appears to be double at the early prophase. At one of the ends of the chromatid there is a true terminal kinetochore and at the opposite end there is a positively heteropycnic piece. One of the chromosomes of the set organizes the nucleolus and all the chromosomes organize an organ at the deeply stained end called *collasome*. The collasome connects the chromosomes into one or several bundles from the telophase to the following anaphase. At metaphase remaining parts of it join the daughter-chromosomes, each of which has its own kinetochore. At anaphase the kinetochores pass to the poles before the rest of the daughter-chromosomes and are connected with them by means of the very thin and elongated kinetochore-necks. Ultimately the collasome-rests connecting the daughter-chromosomes lapse and the entire daughter-chromosomes pass to the poles.

The nature of the orientating mechanism of the kinetochores and some irregularities are discussed. It is pointed out that the kinetochores can function satisfactorily only if their necks are very flexible.

Most cells of the adult animal are $\pm$ tetraploid. The number of chromosomes increases by means of endomitosis, at which an extra division and contraction of the chromosomes occur within the nuclear membrane.

At the first meiotic division there are 9 chromosome configurations. One of them is composed of the sex-chromosomes, *viz.* a large *X* and a small *Y*. At the following telophase the collasome appearing assumes the form of a ring with 18 double chromosome-threads attached to it. This ring is more and more elongated. The chromosomes contract once more to a form resembling that of metaphase chromosomes. This stage is considered a vestigial second meiosis within the spermatids. Once more

there is a resting stage and in the definite spermatozoa the collasomes are clearly visible.

The result of meiosis is not a reduction of the chromosome number but of the number of chromatids constituting a daughter-chromosome. The number of chromatids is altered from two to one.

There are two size-classes of mitotic metaphase chromosomes. The smaller of the chromosomes are supposed to consist of one single chromatid in each daughter-chromosome, whereas the larger chromosomes have double chromatids. There is a tendency to somatic pairing of homologous chromatids two by two in some interphase nuclei, a fact that makes possible a transition from small to large chromosomes with single or double chromatids in the daughter-chromosomes.

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SEED DEVELOPMENT AFTER RECIPROCAL CROSSES BETWEEN DIPLOID AND TETRAPLOID RYE

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INTRODUCTION.

MANY fertilizations result in an abnormal or slow development of the seed ultimately ending in abortion. Embryological investigations have often shown irregular behaviour and disintegration of endosperm and embryo as well as of the somatic tissue of the ovule. Such seed abortion is common after crosses between species with different euploid chromosome numbers, after crosses between a diploid and its autoploids but also after crosses between species with the same chromosome number. In the second of these cases there is only a quantitative difference between the genomes, in the third a qualitative, in the first qualitative as well as quantitative differences. In maize, genes have been found which cause the seeds to cease growing prematurely. Self-sterility may in part be due to irregular seed development.

The literature has recently been reviewed by BRINK and COOPER (1947 b), who have made several important investigations on seed failure. They have found that slow and irregular development of the endosperm leading to breakdown of this tissue is probably the most important feature in the seed abortion. Death of the embryo is more secondary; it is a result of the disintegration of the endosperm. Very remote crosses such as the pollination of species of *Nicotiana* with pollen of *Lycopersicon* and *Petunia* may result in fertilization and normal embryo divisions, though the embryo dies at an early stage owing to weak endosperm (COOPER and BRINK, 1940). Thus, very different gametes may unite, if the pollen tube is able to penetrate to the embryo-sac. To excise such a very young hybrid embryo from the seed and cultivate it on nutrient media is difficult but may sometimes succeed, as is shown by such a strange hybrid as *Hordeum jubatum* × *Secale cereale* (see BRINK and COOPER, 1947 b).

The same authors have observed and studied certain changes in the somatic tissue of ovules of self-sterile *Medicago sativa* after self-fertili-

zation and in ovules of *Nicotiana* and *Lycopersicon* after certain hybridizations. The changes were an abnormally strong development of this tissue and a lack of differentiation in those parts of the chalazal tissue which transport nutriment to the endosperm, the result being a further weakening of the endosperm. BRINK and COOPER have called this kind of seed abortion somatoplastic sterility. It is distinguished by a subnormal growth of the endosperm and a supernormal growth of the somatic tissue of the ovule, death of the embryo being a result of these changes. In some papers the authors seemed inclined to consider the changes in the somatic tissue as prior to and of more importance than the weak development of the endosperm. In a paper of 1947 (1947 b), however, they state ». . . application of the term somatoplastic to this form of sterility is open to criticism on the grounds that it overemphasizes a secondary effect — hyperplasy of the adjacent maternal tissue — rather than what was recognized from the start as the primary factor in the phenomenon, namely, sub-normal endosperm growth». BRINK and COOPER agree with THOMPSON and JOHNSTON (1945) that in the family Gramineae there is no overgrowth of maternal tissues after wide crosses; on this ground the latter authors had ruled out the somatoplastic sterility hypothesis concerning this family. BRINK and COOPER also object to the term embryo lethality (»Embryoletalität») used by FAGERLIND, since the death of the embryo is an effect rather than a cause of the breakdown.

The hypothesis of MÜNTZING (1930, 1933) that purely quantitative relations may be a primary cause of seed abortion has been widely accepted. He pointed out that the relation of the chromosome numbers of embryo, endosperm and somatic maternal tissue is 2 : 3 : 2. If the uniting gametes have different chromosome numbers this relation is altered. »This causes alterations in the morphological (and probably also in the physiological) properties of the tissues concerned and herewith disturbances of their interrelations. The result is poor development or complete abortion of the seeds» (MÜNTZING, 1933, p. 46). WATKINS (1932) accepted, it is true, the hypothesis of the importance of quantitative relations, but considered the chromosome number of the maternal tissue unimportant; seed development depends on a correct quantitative relation between embryo and endosperm. If this relation is greater than the normal 2 : 3 the development is most disturbed. An exclusive importance of the embryo-endosperm relationship has later also been stressed by other authors, for instance, STEPHENS (1942) and HOWARD

(1947). MÜNTZING has, however, raised important objections to such a view (MÜNTZING, 1933).

An influence of the sperm on the seed development other than the fertilization effect has been assumed by KIHARA and NISHIYAMA (1932). They found differences in seed development in reciprocal species crosses in *Avena* and concluded that »from these facts we may assume that the stimulation of male nuclei with lower chromosome number or of nuclei from remote species is weak». When the male nucleus has a multiplicate chromosome number, the stimulus is increased and may be too strong and result in abnormal divisions in the endosperm. MÜNTZING rejects this stimulation hypothesis. The differences between reciprocal crosses can be explained on the numerical relation hypothesis, these relations being different in the cross high × low chromosome number and in the cross low × high chromosome number; no direct evidence of such a stimulation from the male nucleus is known (MÜNTZING, 1933). BRINK and COOPER (1947 b), too, have raised objections against the interpretations of KIHARA and NISHIYAMA. LEDINGHAM (1940), however, who has studied reciprocal species crosses in *Medicago*, considers a different stimulation of male nuclei to be of importance.

FAGERLIND (1937) had certain reservations against the hypothesis of MÜNTZING and seemed inclined to prefer the stimulation hypothesis of KIHARA and NISHIYAMA, stressing, however, »es erscheint mir eigentlich unberechtigt die Erklärung in einem einzigen Faktor suchen zu wollen». Later (FAGERLIND, 1944) he stresses the importance of the 2 : 3 : 2 relation and expresses the view that disturbed equilibrium between zygote and embryo, endosperm and maternal tissue causes lethality; this disturbance may be a secondary effect of an abnormal stimulation influence of the pollen tube (and its nuclei) on zygote, endosperm and maternal tissue and of these latter tissues on one another. FAGERLIND adds that certain facts indicate a diminished pollen tube activity in cases of embryo lethality. BRINK and COOPER (1947 b) pointed out, however, that there is often independent variation of the two phenomena; in maize examples of both independent and correlated variation in pollen tube growth and seed development are known.

BRINK and COOPER (1944) observed slow development of the antipodals in embryo-sacs of *Hordeum* fertilized by rye sperms as well as irregular divisions of polar nuclei and the first endosperm nuclei. They conclude that a rye sperm cannot stimulate the antipodals to normal activity and that the irregular endosperm divisions are an effect of the

weakness of the antipodals. This rather special stimulation effect of the sperms could not be confirmed by THOMPSON and JOHNSTON (1945).

The degeneration of the endosperm plays a very important rôle in seed sterility. It has, however, been pointed out that the importance of endosperm degeneration may be different in different angiosperm families. Many have seeds with a more or less inconspicuous endosperm, »non-endospermous seeds». In such cases irregularities in its development ought to have a less disastrous effect.

Rather few of the reciprocal crosses between a diploid and its autoploids have been submitted to an embryological investigation. They are *Datura Stramonium* (SANSOME, SATINA and BLAKESLEE, 1942) and *Lycopersicon pimpinellifolium* (COOPER and BRINK, 1945). In each, the development after reciprocal pollinations between $2n$ and $4n$ plants was studied. FAGERLIND (1937) investigated reciprocal hybridizations between intraspecific types of *Galium Mollugo* using $2n$, $4n$ and $6n$ types. We have compared seed development in kernels of *Secale cereale* after the artificial pollinations $2n \times 2n$, $2n \times 4n$, $4n \times 2n$ and $4n \times 4n$. The material used was »Stålråg» and tetraploid »Stålråg», produced by Professor MÜNTZING. We have also investigated open-pollinated tetraploid rye. It is a well-known fact that even diploid rye often shows a considerable reduction in the degree of seed setting, the corresponding autotetraploid being of course still more sterile. The development of selfed and crossed kernels of diploid rye has been investigated by LANDES (1939), the main stress being placed on seed abortion. The normal seed development of rye has also been studied by BAYLISS (1940), and some observations are communicated in the paper of BOYES and THOMPSON (1937).

THE SEEDS OF OPEN-POLLINATED TETRAPLOID RYE.

MÜNTZING (1946) has studied the sterility of diploid populations. Differences in seed setting in open-pollinated rye populations may be caused by genotypical or environmental factors. Genotypical factors perhaps account for the fact that seed setting in »Östgöta gråråg» is much lower (about 65 %) than in »Stålråg» (about 80 %), though the pollen sterility is about the same. Cytological aberrations are not rare and »it is not far-fetched to assume that sterility in rye and other allogamous plant species is largely due to structural chromosome variation». MÜNTZING thinks that in such a case the sterility may be haplontic, but that it may also be diplontic.

Seed setting in tetraploid rye is lower than in diploid rye. The difference may, however, not be very large, »the degree of seed setting of the best tetraploids is about 25 % lower than in the diploids» (MÜNTZING, 1949), thus in tetraploid »Stålråg» about 60 %. The chromosome number of tetraploid rye is somewhat variable, hypoploid and hyperploid plants occur; the latter have 29, very rarely 30 chromosomes. Such a variation is common in autoploids and is a result of the formation of multivalents at meiosis. It has been shown (MÜNTZING, 1943) that shrivelled seeds of tetraploid rye give a higher proportion of plants with aberrant chromosome numbers. Selected »very good seeds» gave 16,16 % aneuploids, »very bad seeds» 42,50 %. In accordance with his hypothesis that disturbed quantitative relations between the chromosome numbers of embryo, endosperm and maternal tissue cause seed abortion, MÜNTZING concludes that even the slight chromosomal variation in an autotetraploid may disturb development and influence the quality of the seed. He has also shown that seeds containing a hypotetraploid embryo are on an average less developed than seeds with hypertetraploid embryos. Bad seeds may also result from haploid pollen from a diploid. A number of seeds containing an embryo with 28 chromosomes were, however, also shrivelled.

In 1946 »developed» and »undeveloped» kernels had been collected from four spikes of tetraploid »Stålråg» and four spikes of tetraploid »Vasa II». Judging from the contents of the developed kernels, the collections had been made 10—14 days after anthesis. The developed kernels of »Stålråg» contained cellular endosperm, but in most cases starch had not yet accumulated in the endosperm cells. In some kernels parts of the endosperm were abnormal, lacking cell walls and having small, condensed nuclei: it seemed that parts of the endosperm might have degenerated after a previous normal development. The embryo was elongated but showed no differentiation. The undeveloped kernels had very different contents. These were often deformed and dried, but in other kernels the ovule had an embryo-sac with polar nuclei, degenerating egg-apparatus and antipodals; such an embryo-sac was very probably unfertilized. A few had an enlarged ovule with large antipodals and antipodal nuclei but showed no trace of polar nuclei, endosperm or embryo: the embryo-sac had perhaps been fertilized but further development been interrupted. Many »undeveloped» kernels had some endosperm. A small number of endosperm nuclei in a sac of cytoplasm indicated a very slow or early interrupted development; a comparative high number of free endosperm nuclei (about 250) lying close in a small

embryo-sac and ovule indicated that the normal increase in size of the maternal tissue had failed. Larger »undeveloped» kernels had more endosperm, tissue filling a part of the seed. Degenerating or weak endosperms with condensed nuclei, or with fusions of nuclei occurred. In such weak endosperms mitosis may be disturbed: in a region with numerous anaphase stages, bridges and acentric fragments were very frequent. Undeveloped kernels with endosperm also contained an embryo, which was smaller than in the developed kernels. Sometimes the embryo consisted of only a few cells, with little cytoplasm; the mitotic activity had probably ceased in such an embryo.

The »developed» kernels of tetraploid »Vasa II» showed a more advanced development, the largest part of the endosperm being full of starch, and the embryo showing early differentiation. The degeneration in the »undeveloped» kernels had proceeded further. Many showed no trace of endosperm or embryo or had small remnants of endosperm. Two kernels lacking endosperm had a spherical embryo containing embryonic cells that were large and very poor in cytoplasm, but one of them had a meristematic part with some divisions; 21 chromosomes were counted, fertilization had thus been performed by a haploid pollen tube. Endosperm had no doubt been formed in these two kernels but had degenerated and completely disappeared (compare below). Some undeveloped kernels showed free endosperm nuclei, though in small numbers indicating slow but regular development.

The kernels had been collected too late to allow of a view being formed as to the different forms of seed abortion that occur in tetraploid rye. New collections, therefore, were made in 1949, this time six days after anthesis, and from a population growing at a great distance from populations of diploid rye. The kernels were classified as large, medium or small.

In the large kernels the endosperm was partly transformed to a tissue. The narrow part of the embryo-sac which contains the embryo (the »neck») was filled with cells, and the large central vacuole was lined with two layers of cells at the chalazal side, one layer at the other sides of the ovule. Sometimes the endosperm was less advanced, still containing free nuclei, only in the neck were cell walls formed. The size of the embryo was different, 12—24 cells, 20 was the most common number of embryo cells.

The small kernels had very variable contents. A few had darkly stained remains of macrospores or of an embryo-sac mother cell in the nucellus; no embryo-sac had been formed. Considerably more numerous

were kernels with an unfertilized or degenerated embryo-sac. In certain kernels the embryo-sac had been fertilized, but the subsequent development was very slow or interrupted at an early stage. As instances the following may be given: a small embryo-sac with egg-cell and two large endosperm nuclei, sixteen endosperm nuclei, 64 endosperm nuclei, 80 endosperm nuclei and four-celled embryo, a similar number of endosperm nuclei and eight-celled embryo. Small irregularities may occur: in an endosperm at the 32-nucleate stage some nuclei were small and condensed, and probably incapable of further division, while two metaphase figures with the elongated chromosomes clumped together were found in the vicinity of the antipodals; an embryo was present. The normal time relation between embryo and endosperm growth may be upset: the egg-cell was still undivided in an ovule with 64 endosperm nuclei, on the other hand, an embryo that consisted of eight cells was accompanied by only a few large, irregular endosperm nuclei. Absence or rarity of cell divisions in the nucellus showed the weakness of the ovules of these small kernels, which later very probably abort completely.

In most medium-sized kernels the development was normal though less advanced than in the large ones: the formation of cells had not started in the endosperm, the embryo had only 6—10 cells. One kernel had a large number of free endosperm nuclei, but the embryo was only two-celled. One kernel had well developed endosperm tissue in the micropylar part of the ovule, which also contained a comparatively large embryo; in the other parts of the ovule the endosperm was very weak, consisting of a few free nuclei. One endosperm was completely cellular and had already single starch grains: it had no central vacuole and was shrunken; the embryo was large, consisting of about forty cells. Perhaps fertilization with a haploid gamete had occurred in this case, in the cross $4n \times 2n$ the endosperm usually has this appearance after six days.

The seed sterility of tetraploid rye does not depend on absence of an embryo-sac in the ovule; haplontic sterility seems to play a rather insignificant rôle. It seems that failure of fertilization is much more important. Disturbed development after fertilization must often lead to seed abortion. This is clear from a comparison of the small or undeveloped kernels of »Stålråg» after six days with later collections and with the kernels of »Vasa II» showing still more advanced degeneration. The development of the endosperm is sometimes normal but very slow, only few nuclei being present at six days or even later. This cannot depend on very late fertilization but rather on a weakness of the endosperm. Mitotic irregularities are not conspicuous in earlier stages of

endosperm development though they may occur. Interrupted development is perhaps sometimes due to insufficient nutrient supply to the ovule. The development of the embryo may also show weakness, sometimes independently of the state of the endosperm.

Parts of endosperm tissue in a more advanced state of development may degenerate, in this way shrivelled but germinable seeds no doubt are formed. In some cases bad but germinable seeds perhaps arise in another way: endosperm is from the beginning formed only in the micropylar part of the ovule. KIHARA and NISHIYAMA (1932) believe in a regeneration of degenerated endosperm, new tissue being formed from endosperm still persistent in the antipodal region. Such a regenerate endosperm has not been observed by us.

SEED DEVELOPMENT AFTER 2n × 2n, 2n × 4n, 4n × 2n, AND 4n × 4n POLLINATIONS.

Very occasionally matings between diploid and tetraploid rye result in triploid plants. The junior author obtained in $2n \times 4n$ from 1275 fertilized flowers only 36 well developed kernels; 17 of them germinated and 4 plants were triploids, the other diploids (unsuccessful crosses). In the reciprocal cross $4n \times 2n$ from 783 fertilized flowers 20 well developed kernels were formed, 6 germinated and 4 triploid plants were obtained (the other two were tetraploid).

1946.

In 1946 investigations of a more preliminary nature were made. Only reciprocal pollinations between the diploid and tetraploid type were performed. The kernels were collected from 15 to 96 hours after pollination. Some results of the investigation are given in Tables 1 and 2. Table 1 shows the contents of ovaries collected during the first day. The number of unfertilized embryo-sacs is rather high, particularly

TABLE 1. *Ovules fixed at 15—24 hours after pollination. 1946.*

Cross	E.S. lacking	E.S. unfertilized	E.S. fertilized	n
2 × 4	3	8	42	53
4 × 2	0	16	45	61

TABLE 2. *Fertilized embryo-sacs. 19 hours after pollination. 1946.*

Cross	Polar Nuclei	P.N. dividing	Endosp. Nuclei	n
2 × 4	2	7	15	24
4 × 2	5	15	7	27

when the tetraploid is mother. The majority of ovaries, however, contain an ovule with a fertilized embryo-sac. Only few ovules lack an organized embryo-sac. In Table 2 the stage of development 19 hours after pollination is recorded. In a surprisingly high number of embryo-sacs the polar nuclei were dividing (late prophase—early telophase). This probably indicated that the first endosperm division was rather slow. The development was on an average slower in $4n \times 2n$ than in $2n \times 4n$. This difference between the reciprocal crosses still persisted after 96 hours.

In most cases $4n \times 2n$ kernels after 96 hours had only 32 endosperm nuclei. The egg-cell had as a rule divided once, or was dividing. However, one could find a two-celled embryo together with an endosperm having 8, 16, 32 or 64 nuclei. Also in $2n \times 4n$ very slow development occasionally occurs, the number of endosperm nuclei after 96 hours could be as low as four. In contrast, once, an undivided egg-cell was found together with an endosperm with many nuclei. At 96 hours, disintegrations had not begun.

The weather conditions were very unfavourable at the time of pollination and during this early phase of development. This probably explains the very slow development in the kernels fixed in 1946.

1947.

The experiences from the preliminary investigations indicated a very slow development. The earliest collections were therefore not made until 10 hours after pollination. Collections were then made at 15, 19, 24, 48, 96 hours and after 6, 10, 14 and 21 days. Flowers of two spikes from different plants were emasculated and pollinated artificially, the kernels of each being later collected separately. Thus, the stage of development at each time-interval after pollination was estimated from only two plants; however, from each of the four cross combinations 20 plants in all have been investigated, the observations made at different times often supporting and completing each other.

The young embryo-sac undergoes certain changes before it is mature. It widens but its micropylar part remains narrow, this part of the cereal embryo-sac being called the neck or the pocket. The polar nuclei, which come in contact near the antipodals, move into the neck, enlarge and take a position near the egg-apparatus. An increase occurs in the number of antipodals, which are uninucleate. The embryo-sac becomes campylotropous, the chalaza with the antipodals taking a lateral position. The diploid embryo-sac of the tetraploid type is larger

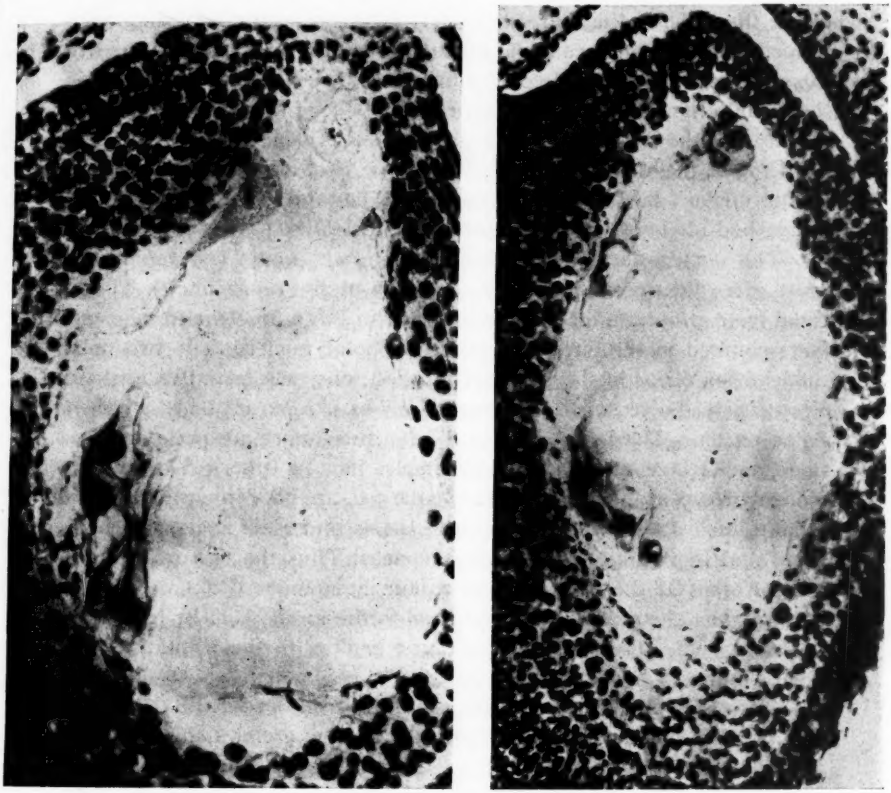

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Fig. 1. $4n \times 4n$. Ovule with fertilized embryo-sac. Male chromatin is visible in the nucleus of the egg-cell, polar nuclei are in division, the antipodals are vacuolated. — $\times$ 175.

Fig. 2. $2n \times 2n$. Abnormal embryo-sac containing three groups of antipodal cells. In an adjacent section were a complete egg-apparatus and two newly formed endosperm nuclei. — $\times$ 175.

and contains larger cells. Polar nuclei as well as the nuclei of the egg-apparatus have, however, only one nucleolus as in a haploid embryo-sac.

10 hours—24 hours. — Table 3 shows the development during the first 24 hours after pollination. In all four combinations the embryo-sacs are as a rule fertilized after 10 hours. The pollen tube may for a short time be observed in the very narrow micropylar canal and be-

tween the cells of the nucellar epidermis. A large number of spindle-shaped starch grains are brought into the embryo-sac with the pollen tube. These grains are visible for some time. One synergid is changed in the usual manner. The fertilization of the egg-nucleus occurs very rapidly, but the chromatin of the sperm is long visible in the egg-nucleus close to the membrane (Fig. 1). Before the first division, however, the zygote nucleus becomes homogeneous. It has only one nucleolus; the sperm had obviously not contained any nucleolus.

The fertilization of the polar nuclei often occurs less rapidly. In most cases the sperm penetrates into one of the polar nuclei. This is clear from prophase stages, for instance in $2n \times 2n$, 14 chromosomes were counted in the one, 7 in the other, polar nucleus. The two polar nuclei often differ in size after fertilization; one may have two nucleoli. Very rarely, however, the sperm is seen as a separate body within a polar nucleus. The term »sperm» is often no longer adequate, because male nuclei in contact with polar nuclei have a spherical or slightly elongated appearance; they become enlarged, are poor in chromatin and form a nucleolus. If fusion is delayed the second male nucleus becomes more and more similar to a polar nucleus. Thus the two male nuclei are different at the time of fertilization. Sometimes the male nucleus accompanies the polar nuclei as far as to the antipodals. In such cases so-called triple fusion probably occurs, leading to the formation of a central nucleus or a spindle.

The polar nuclei perform certain movements in fertilized embryo-sacs. Before the entrance of the pollen tube they are at a short distance from the egg-apparatus, but now they approach the egg-apparatus, both lying at the same level quite close to the boundary of the cytoplasm of the embryo-sac. It looks as if they were attracted by the male nucleus, but perhaps this movement is caused by the pollen tube instead: it was observed in an embryo-sac with a penetrating pollen tube but still no free male nuclei. The polar nuclei, possibly accompanied by the male nucleus, then move in the opposite direction towards the antipodals. The first division may occur before the polar nuclei have reached the antipodals, but it may also occur close to the antipodals in the angle between the antipodals and the wall of the embryo-sac. The first division was often good and 21, 28, 35 and 42 chromosomes could be counted as expected in the different matings. At pro-metaphase the chromosomes may form two different groups, the chromosomes from the two nuclei lying separately (Fig. 3).

Only a small number of the ovules lacked an embryo-sac. They

contained the darkly stained remains of an embryo-sac mother cell or of macrospores.

Abnormal embryo-sacs may occur. Two diploid embryo-sacs (cross $4n \times 2n$) had four large polar nuclei lying in contact, an egg-cell and antipodals; synergids were lacking, the corresponding nuclei had remained free and been enlarged as ordinary polar nuclei. These embryo-sacs were unfertilized. Sometimes one observes small embryo-sacs which have not undergone the usual changes in size and form, the antipodals are unusually small. Such embryo-sacs perhaps cannot be fertilized. A strange haploid embryo-sac had two or three antipodal groups. There was, besides the ordinary group at the chalaza, an extended group between the chalaza and the egg-apparatus and a further group on the other side of and next to the egg-apparatus at the micropylar canal (Fig. 2). The two latter groups were connected through some scattered antipodals. Instances of micropylar antipodals are rare; the senior author has observed such ones in amphi-apomictic *Poa alpina*, where they had been formed through a transformation of cells of the egg-apparatus (HÅKANSSON, 1944). However, this was not the case here, the egg-apparatus was complete; a normal fertilization had occurred and the first endosperm division had just been completed.

In a type of abnormal embryo-sac that could not be fertilized the embryo-sacs were small, and had unusually long necks; the wide part was filled completely with the antipodals, polar nuclei could not be detected. Ovules of tetraploid rye had sometimes a small embryo-sac that had died before it had arrived at the eight-nucleate stage. These latter ovules were instances of haplontic sterility, probably caused by a more irregular meiosis.

Thus, no doubt irregular embryo-sacs that cannot be fertilized occur, but they seem to play a minor rôle in the general sterility of the different types. However, it is not out of the question that one may find individual plants at least in inbred rye with a high percentage of deficient embryo-sacs causing high sterility, as KATTERMANN found in barley (KATTERMANN, 1939).

Failure of fertilizations after entrance of a pollen tube into the ovule is very rare. In one or two such cases sperms were not visible; they were perhaps concealed in the changed synergid. On the other hand, sperms may be observed in the vicinity of the egg-apparatus of an embryo-sac with fertilized polar nuclei and egg-nucleus; in such cases presumably a second pollen tube had entered the embryo-sac.

Table 3 records the ovaries collected during the first 24 hours.

TABLE 3. *Ovules of rye. 1947.*

Time between poll. and fix.	Cross	E.S. unfertilized	Fertilized polar nuclei	P.N. dividing	2 end. n.	2nd div.	4 e. n.	8 e. n.	n
	2 × 2		1	13	2	1			17
10	2 × 4	1	2	17	1				21
hours	4 × 2	3	16	2					21
	4 × 4	2	2	9	5				18
	2 × 2		1	1	2				4
15	2 × 4	1	4	1	8				14
hours	4 × 2	2	2	4	5				13
	4 × 4	3	2	2	9				16
	2 × 2	1	1		11				13
19	2 × 4	1	2	1	6				10
hours	4 × 2	1	1		8				10
	4 × 4	2	3	1	6				12
	2 × 2	3		4	6	7			20
24	2 × 4	3	1	2	5	5			16
hours	4 × 2	1	2	2	10	2	1	1	19
	4 × 4	1			8	3	1		13

Under the heading, »Fertilized polar nuclei», polar nuclei in contact with a male nucleus or central nuclei are also recorded. It seems to be difficult to establish differences between the four combinations concerning time of fertilization. At 10 hours the embryo-sacs were fertilized, the polar nuclei already dividing in $2n \times 2n$, $2n \times 4n$ and $4n \times 4n$. In $4n \times 2n$, male nuclei were seen close to the polar nuclei, or had just completed fertilization, while in $4n \times 4n$ the development had proceeded further. This seems to indicate a slower growth of haploid compared with diploid pollen tubes in tetraploid tissue. This conflicts with the result obtained in the following year and can probably be attributed to chance. One would expect the reverse to be true. In the ovaries investigated 15—24 hours after pollination there were no sure differences between the four combinations. A special rapid development in $2n \times 4n$ similar to that reported from low chromosome × high chromosome species crosses in *Avena* and *Triticum* was not observed. After 24 hours there were still fertilized embryo-sacs with undivided polar nuclei, at any rate in the reciprocal matings.

Clearly, the differences between the four matings cannot be great or are in any case very difficult to prove at such early stages. The difficulty depends largely on the heterogeneity of the material. The

ovaries of the two plants of a certain combination collected at the same time after pollinations often had embryo-sacs of different appearance. Heterogeneity is to be expected in an allogamous species such as rye. The development was more rapid than in the preceding year. After 19 hours the polar nuclei were divided, and the two endosperm nuclei had had time to move rather far apart.

The antipodals seem to enlarge more in $2n \times 4n$ than in $2n \times 2n$, but less in $4n \times 2n$ than in $4n \times 4n$. The rapidity of their transformation and the great variation within the same mating made a more exact determination of the differences impossible.

TABLE 4. *The endosperm after 48 hours. 1947.*

Number of nuclei / Cross	0	2	4	4 div.	8	8 div.	16	16 div.	32	n
$2n \times 2n$					1		4	3	14	21
$2n \times 4n$					2	2	10	2	5	21
$4n \times 2n$	1	1	2	1	7	2	8			22
$4n \times 4n$					4		9		5	18

48 hours. — Table 4 shows the conditions of the endosperm. Development has been slow, not more than 32 nuclei having been formed. The development of the embryo was also remarkably slow: $2n \times 2n$ and $2n \times 4n$ each had only three ovules with dividing egg-cell, or two-celled embryo, $4n \times 2n$ had four and $4n \times 4n$ one. Differences in rate of development of the endosperm are now visible. $2n \times 2n$ endosperm is the most advanced, $4n \times 2n$ has a less advanced endosperm on an average than $4n \times 4n$ and the reciprocal cross $2n \times 4n$.

As a rule the divisions in the endosperm are regular. Giant nuclei were, however, sometimes observed in $2n \times 4n$, indicating irregular mitosis. Some ovules did not possess a multiplied number of nuclei because of failure of some division; such ovules are not recorded in the table. »0 endosperm nuclei» in the table is an ovule containing polar nuclei and two sperms, fertilization had failed. The divisions are simultaneous, as in other cases of nuclear endosperm formation. The divisions commence in the neck. One may, for instance, observe late anaphase stages in the neck, metaphase near the antipodals and in the middle part of the embryo-sac, prophase in the end opposite the embryo. This difference shows that the stimulation inducing the divisions does not come from the chalaza and the antipodals.

The fertilized ovules are increasing in size and undergoing rapid changes. Numerous mitoses are observed in the nucellar tissue on the

chalazal side. The antipodals will soon be halfway between the embryo and the opposite end of the embryo-sac. Close to the antipodals is a group of small chalaza cells. This group may already be distinct after 24 hours; it is more resistent than the surrounding somatic tissue.

96 hours. — There are now great differences of size; many kernels are small.

In $2n \times 2n$ the most common number of endosperm nuclei in well developed kernels was 256, rarer was 128. The neck was in most cases completely filled with cytoplasm and endosperm nuclei. Close to the embryo, cell formation had sometimes occurred, otherwise the endosperm had only free nuclei. The embryo was small, it had 4—7 cells in most cases. Starch from the pollen tube was sometimes still observed. The antipodals, still nearer the embryo end of the embryo-sac, had attained definite size; their nuclei were very conspicuous. The embryo-sac had widened and encroached on the nucellar tissue, though several layers of the latter still persisted. On the chalazal side, however, the somatic tissue was well developed. Small kernels with 32 or only 8 endosperm nuclei and a two- or three-celled embryo showed that the development may be slow or be interrupted.

In $4n \times 4n$ the large kernels had on an average a less advanced endosperm. The most common number of nuclei was 128, several ovules having only 64. A few kernels had a larger number of endosperm nuclei, one had an endosperm at the 512-nucleate stage. The endosperm was, in most kernels, a sac of cytoplasm with one layer of nuclei; the neck was not filled. However, cell walls had been formed in the neck, and the endosperm cells were more clearly demarcated than in $2n \times 2n$. The embryo had 4—5 cells, but larger and smaller embryos of course occurred. More nucellar layers than in $2n \times 2n$ persisted on the abaxial side of the ovule. Many small kernels had an unfertilized embryo-sac.

In $2n \times 4n$ the largest kernels had 256 or less often 128 endosperm nuclei. Intermediate numbers occurred. The development was perhaps only somewhat less rapid than in $2n \times 2n$, but certain differences were observed. Even if the neck is filled with cytoplasm and endosperm nuclei, there is no trace of cell formation. In certain ovules there appeared small vacuoles between the nuclei in the neck, no doubt the first signs of degeneration of the endosperm. Regions with small and condensed nuclei are another sign. Mitotic irregularities are frequent. Bridges may be formed at anaphase, and the anaphase movement may be arrested, causing the formation of a nucleus with a doubled chromosome number. Fusions of nuclei occur, though they are more common

Fig. 3. $2n \times 2n$. The polar nuclei divide close to an antipodal cell. Pro-metaphase, the chromosomes form two groups. — × 1000.

later. Giant nuclei thus arise in different ways, and may be observed in different parts of the endosperm. The irregularities are, however, most common in the neck, and here nuclei completely filled with chromatin were observed. Certain observations show that a multiplication of chromosomes through endomitosis may occur in the sickly neck-endosperm. Giant nuclei were also observed in the $2n \times 2n$ endosperm but much more rarely. The embryo in the larger kernels had about the same size as in $2n \times 2n$ kernels, 4—9 cells. The degree of destruction of nucellar tissue varied. The small-celled chalazal tissue was somewhat isolated owing to a resorption of surrounding somatic tissue. Smaller kernels may show retarded endosperm (for instance, 32 nuclei).

In $4n \times 2n$, as in the preceding cross, there was a great variation. The largest kernels had 128, or rarely 256 nuclei. A certain number had only 64 endosperm nuclei. As a rule the neck was filled more or less with large vacuolated cells; even if the endosperm was only at the 64-nuclei stage, wall formation in the neck had set in; because of the still low number of nuclei, the cells were large and vacuolated. In a small

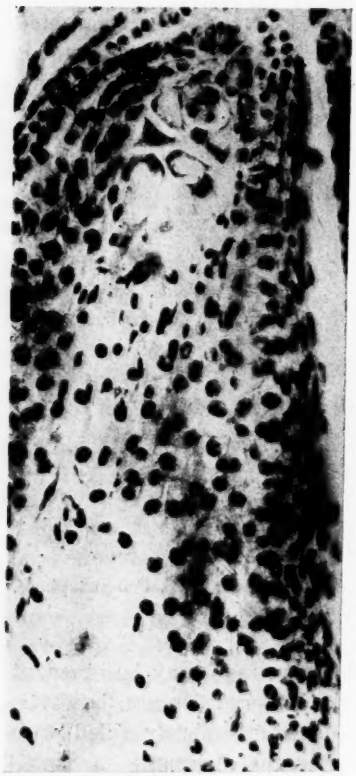

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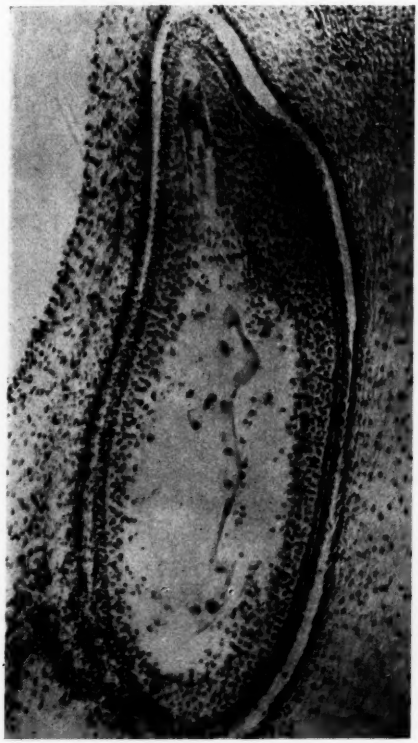
Fig. 4. $4n \times 2n$. 96 hours. Large, vacuolated endosperm cells have been formed in the neck. Anaphase in an apical cell of the embryo. — × 175.

Fig. 5. $2n \times 4n$. Six days. Three giant nuclei in the neck, endosperm nuclei of ordinary size are also visible. — × 175.

kernel a small embryo-sac with only 32 endosperm nuclei had such endosperm cells. Cell walls are no doubt more easily formed than in the other combinations, still more than in $4n \times 4n$. Mitotic irregularities are rare. The average size of the embryos seemed to be four cells. Seed development had been considerably more rapid than in the kernels collected in 1946, when the development no doubt had been exceptionally slow. On the chalazal side of the ovule no somatic tissue had been destroyed; the group of small cells was not isolated. On the abaxial side there were more layers than in $4n \times 4n$. A number of the small ovules



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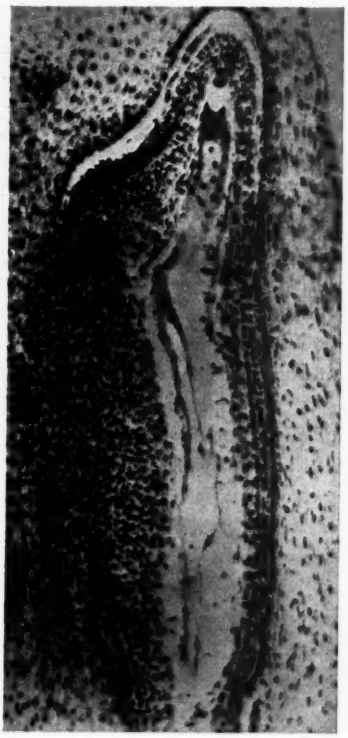

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Fig. 6. $2n \times 4n$. 96 hours. A relatively large part of the somatic tissue of the ovule is destroyed. — × 60.

Fig. 7. $4n \times 4n$. 96 hours. The endosperm is slightly contracted. Mitosis in the endosperm; the most advanced stage — anaphase — is in the part nearest the placenta. — × 60.

had an unfertilized embryo-sac. One was small and had only three antipodals, two of them, however, having two nuclei. On p. 267 it has been pointed out that such embryo-sacs probably cannot be fertilized.

6 days. — In $2n \times 2n$ the most advanced ovules had much endosperm tissue but the central vacuole was not filled. There were more endosperm layers on the chalazal side. Much nucellar tissue was at this side of the ovule, though some had degenerated, but it had disappeared from the top of the ovule and from its abaxial side, the endosperm here

being close to the inner integument. The antipodals also had disappeared. In other rather large kernels the endosperm was less advanced, having two cell layers at the chalazal side and one at other parts, or being cellular only in the neck. The cells at the chalazal side were rich in cytoplasm. Irregularities may occur, patches of endosperm may have small, condensed nuclei, and giant nuclei were observed. Degeneration of the endosperm in the neck was sometimes observed. The embryo was still rather small, it had 30—40 cells but often a smaller number.

Very small kernels had an unfertilized embryo-sac. Some had an enlarged ovule, the embryo-sac was as a narrow fissure, only its antipodals were visible. Probably fertilization had occurred but egg-cell and endosperm nuclei had degenerated.

Between the neck and the antipodals one observes an outgrowth of endosperm tissue towards the attachment of the ovule to the placenta of the ovarian wall. BRINK and COOPER (1947 a) assume a corresponding part of maize endosperm to be an absorbing tissue which transfers nutrients from the maternal tissue to the endosperm. This may well be the case. In $4n \times 4n$ ovules at 96 hours with simultaneous endosperm divisions we observed that the most advanced stages were in the vicinity of the placenta. The mitoses nearer the embryo were somewhat less advanced. This indicates that the stimulating substances reach the endosperm this way (Fig. 7). Weak endosperms show no outgrowth.

In $4n \times 4n$, the endosperm was less advanced and had the appearance described on p. 261. Thus, the central vacuole was always large. The embryo often had 20—24 cells. Antipodals had not disappeared in all larger kernels. The small kernels had an unfertilized embryo-sac or an endosperm with few nuclei.

In $2n \times 4n$, endosperm development had stopped as a rule and there were often signs of degeneration. The endosperm was in most cases one layer of nuclei surrounding the central vacuole; in the very best kernels the endosperm was better developed, at least in certain parts of the ovule. The endosperm on the chalazal side was poor in cytoplasm. In the neck there were still no cell walls. In most endosperms walls are never formed. Irregular and giant nuclei are frequent, mitotic stages are now rarely seen, but fusions are rather frequent. The embryo had a different size (10—30 cells). The relation of endosperm to somatic tissue was very variable. In ovules with comparatively good endosperm large parts of the somatic tissue had disappeared. Antipodals were

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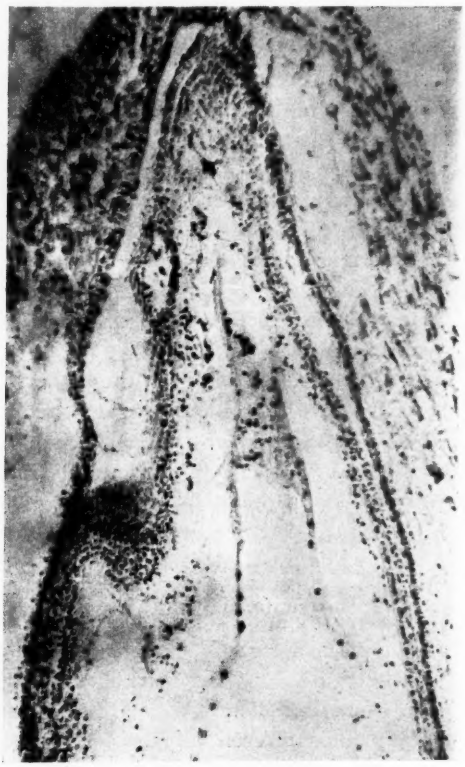

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Fig. 8. $2n \times 2n$. Six days. There are more endosperm layers at the chalazal side of the ovule. — × 60.
Fig. 9. $2n \times 4n$. Six days. Irregular nuclear conditions and failure of cell formation in the endosperm. — × 60.

absent. In some kernels the whole endosperm was in a state of degeneration.

In $4n \times 2n$, the endosperm of larger kernels had the appearance of a loose tissue, the central vacuole was very narrow or filled with tissue. This cannot, however, be interpreted as a sign of a rapid and strong development. Probably the number of nuclei was smaller than in the reciprocal cross or $4n \times 4n$. The large cells in the neck have now divided, the neck endosperm has normal appearance. The endosperm

cells on the chalazal side were poor in cytoplasm (in contradistinction to $4n \times 4n$). There was no close contact between endosperm and somatic tissue, perhaps because the loose endosperm had shrunk (at fixation?). The antipodal nuclei were still seen. Cell-formation occurred in this combination very early, perhaps prematurely. The weakness of the endosperm was also shown by the rarity of mitosis. Smaller kernels had a sac of cytoplasm with still free endosperm nuclei. Mitotic disturbances were rather rare, judging from the absence of giant nuclei. Small, condensed nuclei, however, occurred, showing the beginning of degeneration. Unequal development may occur, certain regions being underdeveloped, others being more normal. A few endosperms had already scattered starch grains, but this was not observed in the other combinations.

In developed kernels the embryo had 15—24 cells but as many as 40 cells were observed. One collection of kernels had many unfertilized embryo-sacs. Other small kernels had 32 or 64 endosperm nuclei. The nucellus was very resistant on the chalazal side, but was often destroyed at the top or the other sides of the ovule. There was on an average more nucellus tissue left than in the other matings.

10 days. — In $2n \times 2n$ kernels the ovule was completely filled with endosperm tissue. The very large cells formed at first in the centre of the ovule had divided. Starch grains were observed in most endosperms. Large nuclei may occur, but more than 42 chromosomes were not observed in any divisions. The embryo consisted of many cells, it had assumed an elongated form but in most cases showed no beginning to any outer differentiation.

In $4n \times 4n$, development was somewhat less advanced. The endosperm was completely cellular, however, and the first starch grains had appeared in some kernels. Many small kernels had been collected, most of them having an unfertilized embryo-sac, still not degenerated.

No kernel from the cross $2n \times 4n$ had an endosperm of normal size. The endosperm was narrow and contained only free nuclei, which may have a healthy appearance. It exhibited a sac of cytoplasm with one layer of nuclei that still persisted although the sac was squeezed between the somatic tissue. Irregular and giant nuclei were still seen, as well as condensed nuclei. Some small endosperms had nuclei of very different size. In some kernels the endosperm was better developed in certain parts, particularly near the embryo end of the ovule. Cell walls had not been formed; starch was never observed in endosperm, but was abundant in the wall of the kernels. The endosperm may be de-

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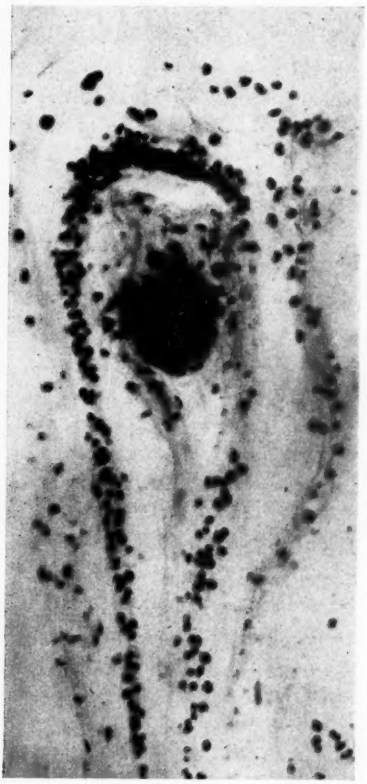

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Fig. 10. $4n \times 2n$. Six days. The central vacuole is nearly filled with endosperm tissue. — × 60.

Fig. 11. $2n \times 4n$. 14 days. Completely undifferentiated embryo. The endosperm has disappeared. — × 175.

generated completely, and kernels with embryo but lacking endosperm occurred. The embryo was less advanced than in $2n \times 2n$, often still being spherical. Much somatic tissue was found on the chalazal side and the top of the ovule in cases where the development of the endosperm had stopped earlier.

In $4n \times 2n$ kernels the endosperm showed variable conditions but was on the whole better than in the reciprocal cross. It formed a tissue of reduced size and irregular outline. Sometimes endosperm tissue was

present in only a part of the ovule. Often the endosperm was very slender but nevertheless cellular and containing starch grains. One kernel had a »normal endosperm» as in $4n \times 4n$ kernels. Most endosperms contained starch grains. Degenerating endosperms without walls were found. A rather large embryo was present in most enlarged kernels. It was perhaps somewhat less advanced than in $4n \times 4n$; it had assumed an elongated form in the best kernels.

14 days. — In $2n \times 2n$ and $4n \times 4n$ there were masses of starch in the endosperm. The scutellum was growing out of the rather large embryo.

The $2n \times 4n$ kernels were thin. Often the endosperm had degenerated; sometimes a small remnant was left or it had disappeared completely. In other kernels the endosperm had not degenerated though it was very small. In a sac of cytoplasm with one layer of free nuclei at the walls a metaphase with 28 chromosomes was observed. Some kernels had a comparatively broad endosperm in the micropylar part of the ovule. It was non-cellular and contained nuclei of very different size and shape. In other cases walls had now been formed in the endosperm. A small embryo was as a rule present in kernels with endosperm. It was elongated but had no scutellum. However, one kernel with a relatively large endosperm showing signs of degeneration had a larger embryo.

The $4n \times 2n$ kernels had in most cases endosperm tissue containing starch. The tissue was, however, as a rule reduced and irregular. Larger endosperms could be found but often had a sickly appearance, many or all nuclei being small and condensed. Metaphases with 35 chromosomes were seen. The embryo was often normal, but in other cases broad and completely undifferentiated. In some kernels endosperm as well as nucellar tissue had disappeared. The embryo was still left, it was spherical and had large vacuolated cells. The outer walls of the epidermis cells were bulged against the interior of the seed; a meristematic still-living part of such an embryo may persist. Many small kernels had an unfertilized embryo-sac. Polar nuclei were still visible, the antipodals were large and contained large nuclei and very large vacuoles. One old unfertilized embryo-sac was unusually large and had numerous single starch grains such as one finds in young endosperm tissue.

21 days. — In $2n \times 2n$ the embryo was fully organized, showing a rapid development during the third week after pollination. Very small kernels lacked embryo and endosperm, some were deformed, other had

antipodals. In $4n \times 4n$ the embryo was very large though somewhat less differentiated than the diploid embryo.

The smallest $2n \times 4n$ kernels had deformed contents. Large though thin kernels had an undifferentiated embryo, which was dead; endosperm was absent or only a small remnant was present. Single starch grains may occur in the endosperm remains. In most kernels, endosperm as well as embryo was absent, having degenerated and then been absorbed. There was one large kernel with rather normal endosperm and normal embryo. In the primary root of the latter 21 chromosomes were counted, which showed that the kernel was not the result of an unintentional selfing.

The $4n \times 2n$ kernels were, on an average, better developed, having more endosperm than in the preceding cross. Some had an endosperm tissue filling a comparatively large part of the ovule. Others had endosperm that was under degeneration, in others again the endosperm had disappeared. The embryo was often in a state of degeneration or was dead. One large endosperm was accompanied by a large but irregular embryo.

1948.

Collections had been made 6, 7, 8 and 24 hours after pollination, unfortunately too late to show differences in the rate of growth of the pollen tube between the four matings. As Table 5 shows, the majority of the embryo-sacs were fertilized after 6 hours; the stage of development seemed to correspond to that after 10 hours in the preceding year. After 7 hours the first two endosperm nuclei were formed. Thus, it cannot be doubted that the fertilization was effected much earlier in 1948. The polar nuclei seem to divide more rapidly; this is also seen from the lower number of divisions observed. This rapidity seems still greater when compared with the observations made on the material collected in 1946. The higher speed of division was no doubt caused by the very favourable weather conditions in 1948, the air temperature being unusually high. A further factor may be that, this year, only ovaries from the middle part of the spike were collected. Observations made after 6 hours indicate a less rapid growth of the pollen tube in $4n \times 4n$ than in $4n \times 2n$; this conflicts with the results obtained the preceding year. It is, of course, not easy to show any differences that may occur in the rate of growth between haploid and diploid pollen tubes in tetraploid tissue. After $4n \times 2n$ and $4n \times 4n$ matings male nuclei were several times observed in the embryo-sac. The antipodals

TABLE 5. *Ovules of rye. 1948.*

Time between poll. and fix.	Cross	E.S. unfertilized	Fertilized polar nuclei	P.N. dividing	2 end. n.	2nd div.	4 end. n.	3rd div.	8 end. n.	n
	2 × 2	1	3	5	5					14
6	2 × 4	3	4	4	5					16
hours	4 × 2	3	6	5	9					23
	4 × 4	5	6	1	2					14
	2 × 2		1		4					5
7	2 × 4		1	1	8					10
hours	4 × 2	2	1		5					8
	4 × 4	3	3	1	5					12
	2 × 2				7	1				8
8	2 × 4		2	1	8					11
hours	4 × 2				7					7
	4 × 4	4	3		8					15
	2 × 2									0
24	2 × 4	6	3	1	2	1	11	1	9	34
hours	4 × 2	4			5		4		12	25
	4 × 4	13			2	1	6	1	3	26

had as a rule already commenced growth in such cases. However, exceptions may occur. An interesting observation was made in $4n \times 2n$. In an embryo-sac with rather small non-vacuolated antipodals the division of the polar nuclei was irregular, there being a bridge at early telophase. This confirms perhaps the hypothesis of BRINK and COOPER of a connection between weakness of the sperm and the antipodals and disturbed endosperm divisions.

Seven and eight hours after pollination the endosperm nuclei were no longer close together, but had moved apart. The collections made after 24 hours also showed the more rapid development in 1948. Coinciding with the observations made after 10 hours, the development was less advanced in $4n \times 4n$ than in $4n \times 2n$. A number of abnormal embryo-sacs have not been recorded in the table.

CONCLUSIONS.

The rate of development of endosperm and embryo in rye is in earlier stages low compared with that in other cereals. *Avena strigosa* and *A. fatua* have much endosperm tissue after three days (KIHARA and NISHIYAMA, 1932); *Triticum vulgare* has the embryo-sac nearly full of endosperm tissue after four days (BOYES and THOMPSON, 1937). In

Zea the cavity is filled after four days (RANDOLPH, 1935); the wide embryo-sac of *Hordeum vulgare* is completely filled after six days (THOMPSON and JOHNSTON, 1945). According to our investigations, the central vacuole in di- and tetraploid rye is filled at a time between six and ten days; LANDES (1939) found the endosperm completely cellular after nine days, while BAYLISS (1940) mentions that the endosperm becomes cellular on the ninth day. BOYES and THOMPSON, who besides their investigation of species and species crosses of *Triticum* also made some observations on rye, state that »at all stages in the material examined development is slower and more variable than in wheat . . .»; abnormalities were observed. The authors therefore suggest that their rye material was not typical, the tardiness and abnormalities perhaps being associated with self-sterility.

The rate of growth of the pollen tube is very rapid in barley, male nuclei establishing contact with the egg-nucleus and polar nuclei after 45 min. (POPE, 1935). PERSIDSKY (1940) states, concerning *Hordeum distichum*, that »the time between pollination and fertilization is somewhat less than one hour». At 30° and 35° C. the male nuclei were in the embryo-sac in 20 min., at 5° C. in 140 min. (POPE, 1947). The great influence of external conditions on the rate of growth of the pollen tube is thus evident. In *Zea* the time between pollination and fertilization is longer, no doubt owing to the very long style. The time is stated as 24 hours (RANDOLPH), 23—28 hours (LOWE and NELSON JR., 1946), 16—18 hours (COOPER and BRINK, 1945). For *Secale* the time of seven hours was given long ago by JOST (1907). Later investigation on seed development indicated slower growth of the pollen tube (SEARS, 1937). We cannot make more exact statements than that fertilization may occur in less than six hours after pollination, and that pollen tube growth is dependent on the weather conditions. It may be added that following mating of *Hordeum jubatum* with *Secale cereale*, rye sperms have reached the embryo-sac after two to four hours, making the fertilization as prompt as after the selfing of *H. jubatum* (BRINK and COOPER, 1944).

The first division of the rye egg-cell was observed in 1947 after 48 hours. BOYES and THOMPSON found at two days »not more than four cells», BAYLISS found the second division on the second day. LANDES records a surprisingly rapid development, two—four embryo cells and 30—40 endosperm nuclei in one day. Our observations show that the rate of development of embryo and endosperm varies; it was different in different years, but perhaps »Stålråg» has an unusually slow

seed development the first days after fertilization. However, there is no noteworthy discrepancy between the observations recorded by LANDES and BAYLISS and our own concerning later stages.

JOST observed reduced pollen-tube growth after selfing. LANDES found an additional cause of self-sterility of rye: seed abortion after fertilization. In seeds fixed five days after pollination or later, irregular endosperm was found. Giant nuclei and irregular nuclei did sometimes occur as well as degeneration of endosperm. Similar disturbances were also observed after cross fertilization, though less frequently; LANDES concluded »thus the irregularities that are present in selfed and crossed rye are alike in their nature and differ merely in the frequency of their appearance». BRINK and COOPER (1947 b) pointed out that some of the endosperm anomalies described by LANDES are artefacts, due to mechanical injury of the young seed previous to fixation. They stress, however, that not all are artefacts; several are similar to those described here. LANDES found that an embryo-sac was absent in only a small percentage of the ovules. More important as a cause of sterility of diploid rye was failure of fertilization: of course this was much more frequent after selfing. LANDES also investigated open-pollinated diploid rye. Many seeds showed endosperm irregularities, in some seeds the endosperm ceased further development at a very early stage. The sterility of this open-pollinated rye was high, 54 %.

In tetraploid rye the rate of development of endosperm and embryo is lower than in diploid rye. This is evident from a comparison of the number of endosperm nuclei and embryo cells, which at corresponding times are less than in the diploid. This is probably due to the autoploid condition (difference in chromosome number), nuclei with the high chromosome number dividing more slowly. No calculation of the volume of the endosperm at different stages has been made. In this respect the tetraploid obviously shows no inferiority, even in these earlier stages, on the contrary the reverse is the case. Cell walls are formed in the neck after 96 hours in tetraploid as well as in diploid rye, though the number of endosperm nuclei is smaller in the former. Autotetraploid *Lycopersicon pimpinellifolium* has a slower development at early growth stages of endosperm and embryo than the diploid type (COOPER and BRINK, 1945, Table 4). At 192 hours, however, the difference is very small in *Lycopersicon*. The time between pollination and fertilization is probably somewhat larger in the tetraploid, though there is small evidence of this. In view of this slow seed development of the tetraploid in the

stages we have investigated, it is of interest that MÜNTZING reports that there is no noteworthy difference in earliness between diploid and tetraploid rye (MÜNTZING, 1948).

The seed sterility of tetraploid as well as diploid rye is only rarely one of haplontic sterility. Our collections from diploid rye also contained a few kernels lacking embryo-sac in the nucellus owing to degeneration of embryo-sac mother cell or macrospores. Such kernels were probably not more frequent in tetraploid rye. They are perhaps rather instances of diplontic than of haplontic sterility. Tetraploid kernels with an embryo-sac degenerated at an early stage of development (two- or four-nucleate stage) are rare; sometimes an organized embryo-sac is formed that shows certain abnormal traits that may make fertilization impossible. These are instances of true haplontic sterility which are still rarer in diploid rye, but certainly play only a minor rôle in the general seed sterility of tetraploid rye. Small embryo-sacs with under-developed antipodals seem to be incapable of fertilization or, if fertilization occurs, the subsequent development is irregular or slow. Such embryo-sac sterility was described by KATTERMANN (1939) in *Hordeum distichum*. KATTERMANN studied the embryology of sterile plants of barley. In one form of sterility (»Sterilitätsfall II«) 42 % of the embryo-sacs had normal egg-apparatus and polar nuclei but the antipodals were subnormal; there were only three cells. It is assumed that one »die Wirkung eines Sterilitätsgens anzunehmen kann, das die normale Entwicklung des Embryosacks hemmt, und welches spezifisch den Antipodenapparat beeinflusst«; a macrospore with the dominant allele undergoes normal development. KATTERMANN observed that in rare cases polar nuclei and egg-cell may be fertilized, embryo but no endosperm being formed (compare also BRINK and COOPER, cited on p. 258, concerning antipodals and endosperm development in *Hordeum*).

An important cause of the seed sterility is failure of fertilization, which seems to occur more frequently in tetraploid than in diploid rye. SANSOME, SATINA and BLAKESLEE (1942) state, concerning tetraploid *Datura Stramonium*, that macrospore abortion is not frequent, the reduced fertility of the tetraploid being in part due to the decreased efficiency of the diploid pollen which leads to failure of fertilization in many ovules and in part to zygotic abortion after fertilization. The seed sterility of tetraploid *Datura* is much larger than that of tetraploid rye. COOPER and BRINK make a similar statement concerning tetraploid *Lycopersicon pimpinellifolium*; »infrequent fertilization appears to be an important factor in the low fertility of the $4n \times 4n$ mating«. It is there-

fore not surprising that increased percentage of non-fertilization is partly responsible for the increased sterility of tetraploid rye.

Still more important, however, are irregularities after fertilization. The post-fertilization irregularities observed by LANDES and ourselves in diploid rye must have a different effect. Some result no doubt in seed abortion, for instance, very slow development at early stages, or degenerations of newly formed endosperm tissue in the largest part of the ovule. Such cases were relatively rare. Other disturbances such as mitotic irregularities or degeneration or faulty development of smaller parts of the endosperm tissue doubtless have less serious consequences. The figures of LANDES show gross disturbances in about one-fifth of the fertilized ovules of crossed diploid rye, but the variety investigated had a rather high seed sterility. In our material of diploid rye gross disturbances were rather rare, they are more common in tetraploid rye. Thus, the increased sterility of tetraploid rye is a consequence of failure of fertilization and of seed abortion after fertilization is more frequent than in diploid rye. Haplontic sterility is of secondary importance.

That rapid increase in size of embryo-sac and antipodals which sets in after fertilization is not shown if fertilization fails. Nor does the ovule undergo the usual changes in shape and size. However, the embryo-sac does not degenerate rapidly. Polar nuclei and antipodals are often distinct after 14 days, the embryo-sac widens slowly, the antipodals, too, gradually enlarge. Mitosis may be still observed in the nucellus after 10 days, but the normal changes of the ovule probably only occur after stimulation. The considerable growth of unfertilized embryo-sacs observed in *Triticale* (HÅKANSSON, 1946) is very rarely observed in rye.

The very large and almost complete seed sterility after diploid $\times$ tetraploid and tetraploid $\times$ diploid rye matings is not due to failure of fertilizations. Such seems to occur about as readily as in the controls, which are artificially pollinated diploid and tetraploid rye. The cause is irregular development and disintegration of the endosperm. On an average there is more endosperm tissue formed in $4n \times 2n$ after 14 and 21 days than in the reciprocal cross, but this tissue is very small and triploid plants have been produced about as rarely from both the reciprocal matings. When the type with the higher chromosome number is mother a better development of the seed is the rule; FAGERLIND (1944, p. 288) found three exceptions, *viz. Galium*, *Rosa* and *Oenothera*. RENNER (1939) noted that in $4n \times 2n$ crosses in *Oenothera* fertilization

occurs but the seeds formed are small and are not able to germinate. In the cross $2n \times 4n$, triploids, so-called *semigigas* plants, are easily formed. In *Oenothera* crosses endosperm and embryo have the same chromosome number, because the embryo-sac has only one polar nucleus.

If one might conclude from the behaviour of the polar nuclei there was evidence from the pollinations in 1946 and 1947 that fertilization is earlier in $2n \times 4n$ than in the reciprocal mating; in 1947 fertilization was later in $2n \times 4n$ than in $4n \times 4n$, the following year an opposite state of affairs prevailed. One would expect a more rapid growth of haploid than of diploid pollen tubes in tetraploid tissue, such as RANDOLPH (1935) proved in *Zea:* in $4n \times 4n$ matings pollen-tube growth was restricted, in $4n \times 2n$ it was normal and fertilizations occurred in a high percentage. But rye may be more similar to *Lycopersicon pimpinellifolium* in this respect. COOPER and BRINK (1945) observed fertilization occurring approximately 24 hours after pollination in diploid flowers, but add that it is »somewhat delayed in the $4n$ flowers regardless of the type of mating». The types in question were $4n \times 4n$, $4n \times 2n$ and $4n \times$ *L. peruvianum*, the latter a diploid species. It is true that several collections of $4n \times 2n$ rye showed a rather high percentage of unfertilized embryo-sacs, but in this case it seems an unsafe conclusion that the pollen tubes are of less importance than the maternal tissue. As the styles are very short and the stigmas large, pollination experiments cannot easily show small differences in the rate of growth of pollen tubes such as probably exist in rye.

A more rapid growth of endosperm has been observed in low × high chromosome crosses than in the controls or the reciprocal cross in species crosses in *Avena* and *Triticum*, as already mentioned. Similarly, LEDINGHAM (1940) found, after a *Medicago falcata* (8) × *M. sativa* (16) mating, more rapid endosperm development than in *M. falcata.* After 32 hours there were often 16 nuclei as against 4 or 8 in *M. falcata.* However, the divisions were irregular. Autoploid crosses show a different picture. In *Lycopersicon pimpinellifolium* the endosperm of $2n \times 4n$ lags behind $2n \times 2n$ from the start, in $4n \times 2n$ seeds the development is slower than in $4n \times 4n$. In rye, differences are difficult to establish immediately after fertilization. At 48 hours a slower development of endosperm in $4n \times 2n$ was manifest. At that time giant nuclei may appear (in $2n \times 4n$). Differences in the rate of development of the embryo are clearer later.

At four and six days there are certain characteristic changes in a

hitherto vigorous endosperm development. In $2n \times 4n$ mitotic irregularities are rather common; in $4n \times 2n$ they are rare. The former cross in this respect somewhat resembles $2n \times 2n$, though irregularities are much less frequent there, while $4n \times 2n$ resembles $4n \times 4n$. The irregularities are most frequent in the neck. A probably important difference is concerned with the formation of cell walls. In $2n \times 4n$, cell formation is very belated notwithstanding the presence of a large number of endosperm nuclei. In the neck, cells are never formed and in most ovules the endosperm degenerates, having only free nuclei. In contradistinction, cell formation is very early in $4n \times 2n$, walls are observed at the 64-nucleate stage or still earlier, the endosperm being completely cellular and the central vacuole filled after six days. Also in this respect $2n \times 4n$ is more similar to the diploid, and $4n \times 2n$, to the tetraploid rye, *i. e.*, to the mother plants, but the differences are very pronounced also in comparison with the latter.

These pecularities of low chromosome × high chromosome and high chromosome × low chromosome endosperm — common *versus* rare mitotic irregularities and late *versus* early cell formation — probably characterize other similar crosses. Common mitotic endosperm irregularities were observed in low chromosome × high chromosome species crosses in *Triticum*, while in high chromosome × low chromosome crosses mitotic irregularities were absent or rare, except in very wide crosses (BOYES and THOMPSON). Mitotic irregularities are very common after the cross *Avena strigosa* (7) × *A. fatua* (21); from the reciprocal cross such are not recorded (KIHARA and NISHIYAMA). FAGERLIND has reported mitotic endosperm irregularities and fusions after intraspecific low chromosome × high chromosome crosses in *Galium Mollugo*. The case of *Medicago falcata* (8) × *M. sativa* (16) has been mentioned. In $2n \times 4n$ matings of *Lycopersicon pimpinellifolium* mitotic irregularities, however, were not observed, though they were common in the species mating $2n$ *L. pimpinellifolium* (12) × *L. peruvianum* (12).

After low chromosome × high chromosome matings late cell formation has been recorded in *Triticum monococcum* (7) × *T. Spelta* (21); cell formation in *T. dicoccum* (14) × *T. vulgare* (21) endosperm is slower than in the reciprocal cross. Early cell formation is sometimes recorded, but always in high chromosome × low chromosome crosses. In *Avena strigosa* and *A. fatua* the first cells are formed in the neck after 72 hours, but in the mating *fatua* (21) × *strigosa* (7) after 48 hours »the cell wall has already been formed in the endosperm although its development is very weak». From the combination $4n \times 2n$ *Galium*

Mollugo, FAGERLIND (1937) reports early cell formation: »Wände werden früher angelegt, als ich jemals bei einem Galieen-Repräsentanten beobachtet habe». *Datura* and *Lycopersicon* have *ab initio* cellular endosperm; the only autoploid crosses investigated more closely therefore cannot give evidence of differences in the time of wall formation in reciprocal crosses.

After 4—6 days the development of the endosperm slows down or stops, the number of nuclei in most ovules does not increase. Only few kernels show a larger endosperm. In $4n \times 2n$, endosperm tissue with starch is formed in many ovules though it has a reduced size.

The development of the embryo is more rapid in $2n \times 4n$ than in the reciprocal mating; later the development stops. The embryo lags far behind at 14 and 21 days, more than in $4n \times 2n$. We have observed living embryos in kernels with degenerated endosperm, and mitosis in a meristematic part of embryos in kernels completely devoid of endosperm and nucellar tissue. One gets a strong impression that the death of the embryo is an effect of the weak development or degeneration of the endosperm.

Observations of seed development after matings between rye and species of *Triticum* and *Hordeum* have been made by some investigators. BOYES and THOMPSON report that in *Triticum vulgare* (21) × *Secale cereale* (7) endosperm development is normal but slower than in the mother; in many cases it is nearly normal, in others there occurs cessation of growth. Cell formation is later than in *T. vulgare*. The authors stress that the result is not to be considered as typical of wheat-rye crosses, because the variety of wheat used (Chinese) crosses as readily with rye as with other wheat varieties. Using another wheat variety as mother the investigators could only report failure of fertilization. MÜNTZING has observed that the kernels after successful crosses are small and usually shrivelled. This indicates disturbed endosperm development. *Secale cereale* (7) × *Triticum vulgare* (21) (Chinese) resulted only rarely in fertilization; in 9-day material two ovaries had undoubtedly developed after cross fertilization, »because the embryo was much smaller than in rye, . . . the endosperm was still entirely noncellular». The neck in one case contained only a single, very large irregular nucleus. These observations of the endosperm conform with our own on $2n \times 4n$ rye matings.

After the mating *Hordeum jubatum* (14) × *Secale cereale* (7) the division of the polar nuclei is only slightly belated. It is, however, sometimes irregular; in the following endosperm divisions, mitotic irregular-

ities are very common; the endosperm degenerates early. In *Hordeum vulgare* × *Secale cereale* crosses (COOPER and BRINK, 1944; THOMPSON and JOHNSTON, 1945) the polar nuclei divide as soon as, or a little sooner than, in selfed material but even the first endosperm division is irregular and very large endosperm nuclei are formed. The development of the endosperm is very abnormal and cells are never formed. The development of the embryo is normal though slow, and stops after the degeneration of the endosperm. Matings between *Secale* and *Hordeum* have only succeeded in one direction. With rye as mother plant, fertilization always fails.

The nucellar tissue is destroyed in older seeds. In ovules with more vigorous endosperm development, the destruction of nucellus tissue proceeds more rapidly. This tissue is most developed at the chalazal side of the ovule, particularly in the vicinity of the placenta of the ovary wall. Numerous cell divisions are long observed here. Evidence of a digestive activity from the endosperm was most clear in $2n \times 2n$. The weak young endosperm tissue of $4n \times 2n$ was least active, while in $2n \times 4n$ varying conditions occurred in this respect. The young endosperm was less differentiated in the two reciprocal crosses. In diploid and tetraploid rye the endosperm on the chalazal side at six days consisted of cells with dense cytoplasm. This was not the case with $4n \times 2n$ endosperm, which is very poor in cytoplasm, or the non-cellular endosperms of $2n \times 4n$. The outgrowth in the direction of the placenta was less conspicuous in the reciprocal matings, probably owing to the weakness of the endosperm.

The seed development is sometimes better and exceptional kernels show a rather normal development. However, the reciprocal crosses have, as it seems, not only the seed sterility of diploid and tetraploid rye to a greater extent, but a further superimposed sterility, no doubt owing to disturbed quantitative relations. Some evidence of this is afforded by the change of the moment of cell formation in the endosperm. There are many similarities between the conditions of the seeds after reciprocal matings of high chromosome and low chromosome species of *Triticum* and *Avena*. COOPER and BRINK (1945) could compare development after interspecific and intraspecific crosses with the same species. They concluded that the course of seed failure associated with matings between the diploid and its autotetraploid is very similar to that following the interspecific cross.

Differences in seed development after reciprocal matings have been observed also in cases when the types have the same chromosome

number. In *Primula vulgaris* × *P. elatior* 85 % of the seeds contain endosperm and embryo which are definitely though poorly developed. These seeds have subnormal size and germination is very poor. In *P. elatior* × *P. vulgaris* 80 % of the seeds lack endosperm and embryo. The remaining 20 %, however, germinate fairly well (VALENTINE, 1947). This difference was as in other similar cases (compare FAGERLIND, 1937) ascribed to the different constitution of the endosperm. In the former cross the endosperm had 2 *vulgaris* + 1 *elatior* genome, in the latter 1 *vulgaris* + 2 *elatior* genomes. VALENTINE assumes that the hybrid embryo does not thrive so well in the former endosperm, and that the difference shows that the two species are not very closely related. JENKIN (1933) has earlier assumed that the difference between the cross *Lolium perenne* × *Festuca pratensis* and its reciprocal is mainly shown in a difference in the development of the endosperm, which is different in constitution in the two crosses.

Embryological investigations of seed development after matings between diploid species have chiefly been made on *Datura* (SANSOME, SATINA and BLAKESLEE, 1942; SATINA and BLAKESLEE, 1935; SACHET, 1947; SANDERS, 1947). Several combinations have been studied by SACHET. As a rule, most ovules are fertilized, but the endosperm degenerates after a different number of divisions. The egg-cell divides at least once; only in *D. pruinosa* × *D. Metel* is division of the fertilized egg-cell very rare. Thus there are certain differences between the different crosses; but SACHET also stresses the great variability in the subsequent development of the hybrid seeds, seeds on the same plant showing different ratios and different patterns of growth and disintegration. The pollen tube is assumed to bring to the ovule certain substances which in incompatible crosses are incompatible with the substances of the ovule. This assumption is extended to $2n \times 4n$ and $4n \times 2n$ crosses, there being too much or too little of certain auxins. The length of the style is also of importance: when certain species having an unusually long style are mother plants some combinations do not result in fertilization. The importance of the style is also evident from SANSOME, SATINA and BLAKESLEE: in $2n \times 4n$ *D. Stramonium* no fertilizations occur. They succeed, however, when the mother plant is a periclinal chimæra, a diploid with a tetraploid epidermal layer. The style is in fact formed from the epidermal layer. SANDERS could not bring about fertilization through graftings in such crosses, where length of style was a hindrance, nor could she by injections of growth-promoting substances induce further development of weak endosperms.

SANSOME, SATINA and BLAKESLEE could not arrive at a definite conclusion in regard to the sequence in which the different tissues of the *Datura* seed begin to degenerate. In species crosses the embryo often begins to disintegrate first, in *intra se* crosses the degeneration mostly appears to have been initiated by the endosperm. The authors point out that disintegrations are more readily observed in the large endosperm cells than in the dense cells of the embryo. They found that the opening at the chalazal end is not closed in aborting seeds, therefore the disintegration of the endosperm is not the result of a cutting off of the nourishment, as in incompatible matings of *Nicotiana* investigated by COOPER and BRINK (1940). SANSOME, SATINA and BLAKESLEE thus take up a rather reticent attitude towards BRINK and COOPER's hypothesis of somatoplastic sterility.

BRITTEN (1947) applied naphthalene acetic acid to maize kernels at the time of fertilization. The maternal tissues, that is pericarp and nucellus, were stimulated to an abnormally rapid growth during a short period. Embryo and endosperm later showed abnormal differentiation as well as retarded development. This seems to give support to the earlier idea of BRINK and COOPER that the weak development of endosperm is caused by excessive growth of certain maternal tissues. BRITTEN stresses, however, that his experiments cannot be considered as critical evidence in favour of the theory of somatoplastic sterility. SWANSON, LAVELLE and GOODGAL (1949) treated ovaries of *Tradescantia* with 2,4-dichlorophenoxyacetic acid one or two days after flowering. In contrast to BRITTEN, they found no strong development of somatic tissue. On the other hand, they observed an inhibition of the development of the endosperm; in relatively mature ovules the endosperm consisted of but few nuclei. The embryo was less affected by the treatment. Somatic tissue of the chalazal region of the ovule disintegrates early, but is not invaded by the inhibited endosperm. The ovules collapse finally. The endosperm seemed quite unable to utilize the food reserves released through the early disintegration of the chalazal tissues, which occurs even more rapidly than in untreated ovules; the death of the embryo seemed to be secondary. Ovules in ovaries treated 8 to 10 days after flowering matured without collapsing.

Like other investigators of seed abortion in Gramineae, we have not observed overgrowth of the maternal tissue. Clearly, however, quantitative relations between endosperm and maternal tissue are of importance. The importance of chromosome number is minimized by MODILEVSKY (1945), who has studied seed development in the cross

Nicotiana rustica (24) × *N. Langsdorffii* (9). He observed different chromosome numbers in the endosperm. Ovules with the normal number of chromosomes in the endosperm (57) develop to germinable seeds, if the embryo is haploid or diploid (through haploid or diploid parthenogenesis). In ovules with 57-chromosome endosperm and a hybrid embryo, however, the endosperm is weakly developed and the embryo degenerates; as a rule such seeds cannot germinate. In ovules with endosperm having fewer than 57 chromosomes the endosperm degenerates early, the embryo continuing development for some time; resulting seeds are small and cannot germinate. The sperm is said (we have only read the English summary) to fuse sometimes with only one polar nucleus; the two polar nuclei divide separately and give endosperm cells with 24 and 33 chromosomes. A parthenogenetic development of endosperm with 48 chromosomes was also observed. MODILEVSKY concludes that these relations between embryo and endosperm cannot be explained solely by the chromosome numbers. »Here we evidently have physiological phenomena of a complex nature.» The embryo-sac should be regarded during the development of embryo and endosperm »as an autonomous system having special functions». However, the quantitative relation of the endosperm to the maternal tissue cannot be neglected, the degeneration of 24- and 33-chromosome endosperm may very well be a result of the much changed relations.

The weakness of the endosperm in reciprocal crosses between diploid rye and its autotetraploid is hardly due to its $4n$ or $5n$ condition but rather to its relation to other parts of the seed. Normally the numerical relation of the chromosome numbers of maternal tissue: endosperm : embryo is in $2n \times 2n$ and $4n \times 4n$ 2 : 3 : 2 and 4 : 6 : 4, respectively, that is 1 : 1,5 : 1; in $2n \times 4n$ the relation is 2 : 4 : 3, that is 1 : 2 : 1,5, while in $4n \times 2n$ it is 4 : 5 : 3, that is 1 : 1,25 : 0,75, using the number of the maternal tissue as unity. The relation embryo/endosperm which is normally 0,67, is 0,75 in $2n \times 4n$ and 0,60 in $4n \times 2n$. Thus in the cross $2n \times 4n$ the endosperm as well as the embryo are »hyperploid» in relation to the maternal plant, and the embryo is also »hyperploid» to the endosperm. In the cross $4n \times 2n$ the endosperm and embryo are »hypoploid», the embryo also being »hypoploid» to the endosperm. Compared with the mother plant there are certain differences between the »hyperploid» and »hypoploid» endosperms which, as was pointed out above, are characteristic not only of rye but seem to be of a more general importance. The »hyperploid» endosperm at 48 hours has developed normally but at four or six days mitotic disturbances are

comparatively frequent and wall formation is belated or in most cases fails. The »hypoploid» lags after 48 hours, mitotic disturbances are rare but wall formation occurs very early. Some of the disturbances of mitosis in the former case are probably a result of the failure of wall formation as, for instance, the numerous fusions in the neck, but all cannot have this cause. The development of the embryo is in these stages normal and, to judge from the observations made, the death of the embryo is secondary to the disintegration of the endosperm. It therefore seems to us that at four to six days the aberrant relation between endosperm and maternal tissue is of more importance than the embryo/endosperm relation. The difference in the time of wall formation may well be a consequence of these changed relations. Cell formation may be dependent on substances produced by the maternal tissue. The »hypoploid» endosperm has at an early stage received a sufficient quantity to induce wall formation, while in the »hyperploid» endosperm an insufficient quantity is present at the proper time of cell formation. The production of haploids in angiosperms supports the importance of the endosperm: maternal tissue relation. In the rare cases when haploid plants arise, the polar nuclei have probably been fertilized and the relation to the mother plant is normal with regard to the endosperm.

In certain cases changed quantitative relations have a less serious influence on the quality of the seed. A rather good fertility is shown by *Populus tremula*, $2n \times 4n$. JOHNSSON (1945) found the diminution in seed production after pollination from the autotetraploid to be 21 %, which must be considered as rather low compared with *Secale*, *Datura*, *Solanum*, and other cases. The reciprocal cross was not made owing to lack of a tetraploid female tree. *Populus* has however non-endospermous seeds which mature very rapidly. The seeds of *Beta* are also non-endospermous. RASMUSSON (1948) states that in sugar beet about 20 % of the plants from $2n \times 4n$ are triploids; in $4n \times 2n$ as many as 80,5—88 % are triploids. Many angiosperm families have non-endospermous seeds; but with the exception of Orchidaceae and Podostemonaceae an ephemeral endosperm is formed in earlier stages, or the endosperm is persistent, but is only an inconspicuous part of the mature seed. Transient endosperms may play an important though unknown rôle in certain stages of seed development, and in such cases disturbed quantitative relation may lead to seed sterility.

MÜNTZING showed that shrivelled but germinable seeds from tetraploid rye more frequently have an aneuploid embryo. It may seem

strange that such small quantitative changes of endosperm/maternal tissue relation are of importance. The shrivelling of germinable seeds is probably a result of disturbances in the endosperm at a later time in seed development. Other factors than the endosperm/maternal tissue relations may be responsible for shrivelling in these later stages, for instance, the chromosome number of the embryo.

In crosses of the type $2n \times 3n$, $3n \times 2n$ and $3n \times 3n$ different results have been obtained in different genera (see LEVAN, 1942). The seeds after such crosses have been studied only in $3n \times 2n$ *Datura Stramonium* (SATINA, BLAKESLEE and AVERY, 1939). Fertilization may occur with egg-cells having 12—24 chromosomes, though the majority remain unfertilized. In many ovules endosperm and embryo disintegrate soon after fertilization, often growth is arrested at later stages. The size difference between the seeds in a mature capsule are great. Most of the plants from defective seeds had extra chromosomes. Diploid seeds were larger than $2n + 1$ seeds, the latter are larger than $2n + 1 + 1$ seeds; certain extra chromosomes in $2n + 1$ embryos caused the seeds to be larger, while certain other chromosomes caused the seeds to be smaller. The junior author has made the reciprocal crosses between diploid and triploid rye. In $3n \times 2n$, seeds with a diploid embryo were not better than seeds with an aneuploid embryo. Regarding weight of kernels there was, however, a significant difference between diploid and 16 chromosomal kernels but no difference between diploid and 15 chromosomal.

Through fertilization the ovules are stimulated to growth. We have not been able to show that the stimulation has been greater in $2n \times 4n$ than in diploid rye, nor that it is less in $4n \times 2n$ than in tetraploid rye at early stages. Perhaps measurements of the size of the ovule made on a large material may give some results. The relation between the chromosome number of the sperm, the changes of the antipodals and division of polar nuclei in newly fertilized embryo-sacs may also be worthy of further investigation. It may be added that even if the numerical relations in the seed are abnormal there must be interaction between maternal tissue and endosperm (and embryo), as is seen from the rather parallel growth of these tissues in most cases. This interaction however meets with difficulties. The difficulties are sometimes overcome and the development of the endosperm is not stopped but may proceed in a rather normal manner with a germinable seed being formed.

SUMMARY.

Reciprocal crosses between diploid and autotetraploid rye result in aborted or shrivelled seeds. Very few triploids are procured.

Fertilization occurs readily in $2n \times 4n$ and $4n \times 2n$, the first development is regular.

At four and six days the endosperm shows differences compared with the controls $2n \times 2n$ and $4n \times 4n$. In $2n \times 4n$ the number of nuclei are about the same as in $2n \times 2n$, mitotic irregularities are, however, more common and cell formation in the endosperm is belated and usually fails. In $4n \times 2n$, on the other hand, the rate of nuclear divisions has slowed down but mitotic irregularities are rare and cell formation is early, also compared with $4n \times 4n$.

Later disintegration of the whole or parts of the endosperm occurs. The arrest of the growth and death of the embryo seems to be secondary to this disintegration. Disturbed relations of the chromosome numbers between endosperm and maternal tissue must be an important factor, though not the only one causing seed sterility.

The increased seed sterility of autotetraploid rye compared with that of diploid rye is due to increased failure of fertilization and increased frequency of slow and interrupted development after fertilization. Haplontic sterility seems to be of less importance.

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MEIOSIS IN TWO DIFFERENT CLONES OF THE APOMICTIC CHONDRILLA JUNCEA

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I. INTRODUCTION.

THOSE apomicts whose legitimate embryo-sac mother-cells (EMC's) regularly give rise to unreduced parthenogenetic embryo-sacs could, until quite recently, be divided into two different groups with regard to the first division of the EMC. The first of these groups is represented by a pure mitotic division, which has been studied in *Hieracium* by the author (1941). The second group is represented by a so-called pseudohomotypic division, which has been studied especially in *Taraxacum* by GUSTAFSSON (1935, and later). As to the latter division, two papers have recently been published by two different workers who have found that GUSTAFSSON's interpretation of the conditions in *Taraxacum* must be erroneous. Thus FAGERLIND (1947 c) and BATTAGLIA (1948), at about the same time and independently of each other, have found that the EMC's of *Taraxacum* divide in a manner very similar to the formation of restitution nuclei in the PMC's of certain *Hieracia*, which ROSENBERG (1917, 1926—27) called contraction nuclei. Consequently, one can also call in question other cases where this division has been considered to occur. Thus FAGERLIND (1947 b) refers to *Erigeron*, which has been studied by HOLMGREN (1919), GUSTAFSSON (1935) and the present author (1944) and in which the pseudohomotypic division has been considered to occur with certainty. Through his own investigations (FAGERLIND, 1947 b) and partly on the basis of certain »deviating» division stages published by the author (BERGMAN, 1944) he comes to the conclusion that here, too, we have another case of a contraction nucleus or, as FAGERLIND calls it, a metaphase restitution nucleus. No doubt FAGERLIND is right in his opinion, and one has to agree with him when he calls in question the presence of a pseudohomotypic division, *inter alia*, in *Chondrilla juncea*, which until now has also been definitely considered to occur.

Until now *Chondrilla juncea* has been the subject of only a few relatively superficial investigations with regard to the divisions in the EMC's. The last one was made by the author, who for certain reasons

could only make a superficial examination at the time (*cf.* BERGMAN, 1944, p. 249). The division stages discovered corresponded very well with GUSTAFSSON's pseudohomotypic division, and therefore it was concluded that probably such a division occurs in *Chondrilla*, too. On account of the papers of FAGERLIND and BATTAGLIA, however, the situation is now changed and *Chondrilla* needs a more profound examination. Such an examination has now been carried out by the author and the results are presented in this paper.

ROSENBERG (1912) was the first to establish on a cytological basis the occurrence of apomixis in *Chondrilla juncea*, but as a matter of fact he says nothing about the first division in the EMC's, as he intended publishing a more detailed report later, which he did not finish. As to the pollen formation, ROSENBERG gives some information which is important for the present work. He says that the PMC's divide only once and therefore only dyads are formed in the microsporogenesis. »Nur selten habe ich Tetraden gefunden» (p. 918). In 1933, along with some other *Chondrilla* species, *Ch. juncea* was again investigated, but this time by PODDUBNAJA-ARNOLDI. She states (p. 71) that the EMC regularly runs through a somatic division but with contracted chromosomes; hence the same type of division as HOLMGREN (1919) described in *Erigeron cfr. annuus* and as GUSTAFSSON (1935, and later) declares to occur in *Taraxacum* and other apomicts and as he calls pseudohomotypic. With regard to the pollen formation in *Chondrilla juncea*, PODDUBNAJA-ARNOLDI (p. 62) points out that ROSENBERG is wrong in his opinion that only dyads are formed, for in her material triads, tetrads and polyads are also present.

The material of this investigation comes from two different places, partly from Hortus Bergianus in Stockholm, partly from the botanical garden of Uppsala University.

At first it was intended only to study the EMC of *Chondrilla*, but it was not long before attention was attracted by the PMC's and their divisions. It was found that in the Stockholm plants the microsporogenesis was nearly always completed with dyads, whereas the Uppsala plants nearly always completed their microsporogenesis with tetrads. Hence, *Chondrilla juncea* appears in two different clones which differ from each other as to their pollen formation, and evidently ROSENBERG happened to investigate individuals of the one clone, whereas PODDUBNAJA-ARNOLDI investigated individuals of the other.

This investigation of the meiosis in the PMC's has shown what was to be expected, namely remarkable differences between the two clones,

and it also gives the cytological explanation of the differences in the pollen formation. Moreover, in the dyad-forming Stockholm plants meiosis in the PMC's and EMC's proved to be identical, which has been of great value for clearing up the course of division in the EMC's. In a Composite there can be many successive division stages in the anthers, whereas each flower has only one ovule with a single EMC. Therefore it stands to reason that it is much easier to reconstruct the course of division in the PMC's, where as a rule it is possible to determine the *succession* of the division stages, too, which is much more difficult in the EMC's. It is therefore appropriate to begin the following account with the microsporogenesis.

II. MEIOSIS IN THE POLLEN MOTHER-CELLS (PMC's).

1. CHONDRILLA JUNCEA FROM UPPSALA.

ROSENBERG (1912) estimated the number of somatic chromosomes in *Chondrilla juncea* at 14—16. Later it was found by PODDUBNAJA-ARNOLDI (1933) and by the present author (1944) to be 15. Root tips from both the Uppsala and the Stockholm plants have been re-examined and the number found to be $2n = 15$. Hence, compared with the sexual *Chondrilla ambigua* ($2n = 10$) (PODDUBNAJA-ARNOLDI, 1933), *Chondrilla juncea* ought to be considered as a triploid.

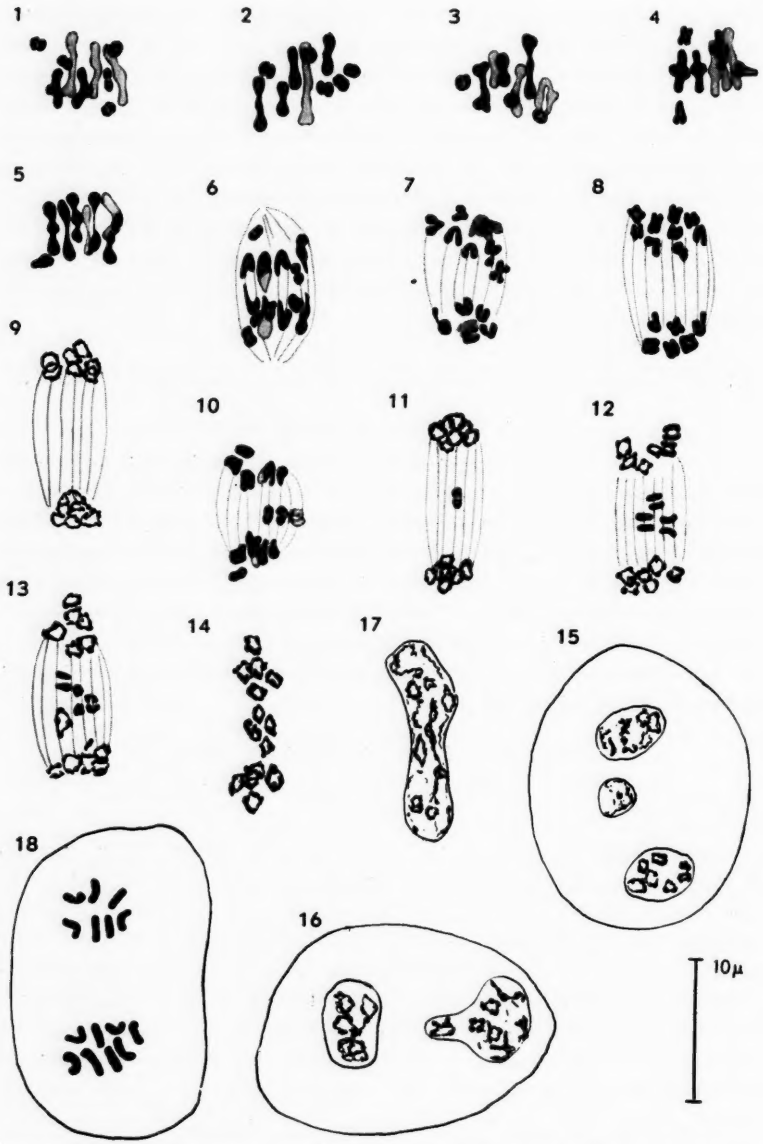
The PMC's of this plant are characterized by an extensive bivalent formation, as seen in Table 1.

TABLE 1. *Extensive bivalent formation.*

Associations	$5_{II} + 5_{I}$	$6_{II} + 3_{I}$	$7_{II} + 1$	Total No. of PMC's
No. of PMC's	7	18	2	27

One would expect five bivalents (possibly trivalents) to be the maximum for this plant, but $6_{II} + 3_{I}$ is more common than $5_{II} + 5_{I}$. In two PMC's even $7_{II} + 1_{I}$ were established. Figs. 1—5 illustrate five metaphases with these associations, $5_{II} + 5_{I}$ being shown in Figs. 1—2, $6_{II} + 3_{I}$ in Figs. 3—4, and $7_{II} + 1_{I}$ in Fig. 5. Evidently autosyndesis occurs in a set of only five chromosomes, which is quite a remarkable fact. However, one has to bear in mind that it is a question of an apomict and that in the course of time structural changes may have taken place which have later been retained through the parthenogenetic reproduction.

On account of the above-mentioned conditions one would expect polyvalents to occur also, but in spite of close studies of suitable stages



Figs. 1—18. *Chondrilla juncea* from Uppsala. — Meiosis in the PMC's. — 1—5, metaphases with different chromosome association. — 6—8, anaphases. — 9, telophase. —

nothing has been found to indicate with certainty the presence of any polyvalent.

The author's interest was especially in the univalents and their behaviour in the two meiotic divisions of this plant, this in order to be able later to make comparisons with the corresponding conditions of the dyad-forming Stockholm plants. The univalents of the latter behave in a peculiar manner that determines the result of their division.

From Table 1 it appears that the number of univalents varies between 1, 3 and 5, three being the most common. At metaphase they are, as a rule, longitudinally divided and are scattered on the spindle at random (Figs. 1—5). Sometimes there may be one located on the equatorial plane and there, like the bivalents, taking up an axial position, which appears from Figs. 1 and 4. At anaphase the bivalents separate into two different groups and pass to opposite poles in the usual manner. When the bivalents separate at anaphase the univalents follow one of two courses: (1) those lying outside the equatorial plane are included in the group of daughter bivalents passing to the nearest pole (*cf.* Figs. 6—9); (2) those lying at the equatorial plane remain there until the resting stage (*cf.* Figs. 10—14). Of these courses the first one is the more common (*cf.* Table 2).

TABLE 2. *Number of lagging univalents at first anaphase.*

No. of lagging univalents	0	1	2	3	4	5	Total No. of PMC's
No. of PMC's	47	36	13	3	1	0	100

Hence, of a hundred anaphases nearly half were without lagging chromosomes and a third had only a single one among them. From these facts it is concluded that the univalents of this plant are not able to move to the equatorial plane during metaphase-anaphase, but remain in their original prometaphase position. It seems more likely that the univalents had the capacity of moving in opposite directions, *i. e.* to one pole or the other. However, this does not apply to the univalents lying at the equatorial plane or in its immediate area. They remain there during the whole anaphase-telophase. During the last-mentioned stage their longitudinal division is completed and they often separate into their daughter chromatids, at least as far as externals go (*cf.* Fig. 12). Looking at Figs. 11—13 one also observes that the lagging chromosomes enter telophase later than those situated at the poles. In

10, anaphase with lagging chromosomes. — 11—14, telophases with lagging chromosomes. — 15, interphase with one micro nucleus. — 16, interphase, one of the nuclei has incorporated lagging chromosomes. — 17, restitution nucleus. — 18, the second metaphase.

the dyad stage they give rise to micro nuclei (Fig. 15) or such figures as in Fig. 16. When the lagging univalents are not few, as in Fig. 13 (where they are four), chances of the formation of a restitution nucleus are present. Such a nucleus is in formation in Fig. 14 and a complete one appears in Fig. 17. However, restitution nuclei are rare in this plant (less than 1 per cent), undoubtedly because the lagging chromosomes in most cases are so few in number (*cf.* Table 2).

From Figs. 10—13 it appears that the lagging univalents divide into their two chromatids, though these are unable to pass to the opposite poles as in mitosis. This is a rule to which no exceptions have been observed. This rule has also been confirmed in studies of metaphases in the second meiotic division (Fig. 18 and Table 3).

TABLE 3. *Chromosome distribution at second metaphase.*

Distributions	7-8	6-9	7-1-7	8-1-6	6-2-7	Total No. of PMC's
No. of PMC's	33	12	7	8	2	62

Thus, in sixty-two PMC's the sum of the chromosomes at the two metaphases (including any lagging chromosomes) has been fifteen. Naturally the chromosome number of the two plates varies, 7—8, however, being the most common, as can be seen in Table 3 (Fig. 18). In the distributions 7—1—7, 8—1—6 and 6—2—7 the central figure refers to the number of lagging chromosomes.

With regard to the division conditions of the univalents, the anaphase of the second meiotic division on the whole confirms the above statements. If in the first meiotic division the univalents divided mitotically, lagging chromosomes ought to occur at anaphase of the second division. However, in sixty-eight such anaphases only two cases with a lagging chromosome were observed.

A study of the completed tetrads showed that they are as a rule normal. Judging from Table 2, a great number of PMC's with micronuclei would be expected, but this is not the case. Probably this depends on the fact that in a great many of the PMC's any lagging chromosomes are incorporated with the dyad nuclei in the manner shown in Fig. 16. Thus, of ninty-two interphases examined, only sixteen were found to have a micronucleus, the rest being normal or looking almost like that pictured in Fig. 16.

Reviewing the observations on the microsporogenesis of *Chondrilla juncea* from Uppsala, we see that meiosis in the PMC's is characterized by an extensive synapsis, most often with $6_{II} + 3_{I}$. The univalents fail

to move to the equatorial plane. Those which at prometaphase were located on this plane complete their longitudinal division but remain on this plane. Later they form micronuclei, or are (rarely) included in restitution nuclei, or in the dyad nuclei (*cf.* Fig. 16). As a rule, the result of the microsporogenesis is normal tetrads.

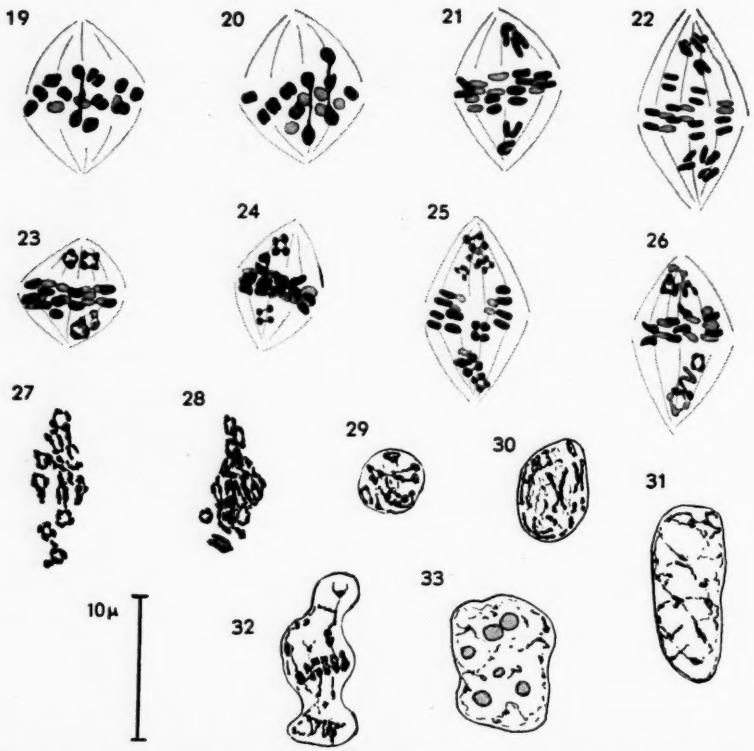
2. CHONDRILLA JUNCEA FROM STOCKHOLM.

The microsporogenesis of these plants differs from that of the Uppsala plants in two essential respects. It is nearly always completed with dyads and is characterized by total or nearly total asynapsis. When synapsis is present it is limited to a few bivalents and, as a rule, only occurs in certain loculi of the anthers. Loculi with total asynapsis and with partial asynapsis are approximately of the same frequency. It may be appropriate to start with the latter type.

In normal sexual Composites, the younger and older stages succeed one another from one end of the loculus to the other. In apomictic Composites, *e. g.*, *Archieracium*, this succession is often interrupted and is only to be found in parts of the loculus. The present material of *Chondrilla juncea* in this respect behaves like normal sexual Composites, only small deviations having been observed. Naturally this has been of great value in the reconstruction of the course of the division.

Figs. 19—33 show a number of PMC's from the same loculus lying successively. Figs. 19 and 20 illustrate the youngest stages and lie at the bottom of the loculus. The bivalent formation appears to be very limited, three being the highest number of bivalents observed. In Fig. 19 one can count $1_{II} + 13_{I}$, and in Fig. 20 $2_{II} + 11_{I}$. With regard to the univalents they are quite well accumulated on the equatorial plane. At the prometaphase, however, they are scattered at random on the spindle and evidently they can therefore move on to the middle of the spindle. Above these two metaphases a row of anaphases lies in the direction of the top of the loculus. Six of these were selected in an upward direction and drawn in Figs. 21—26. As can be seen, the daughter chromosomes of the bivalents have separated and reached the poles. In Figs. 22, 25 and 26 there have been three bivalents, in Figs. 21 and 23 two each, and in Fig. 24 only one. The univalents, on the contrary, have not until now reached the equatorial plane, where they have taken up a metaphase position.

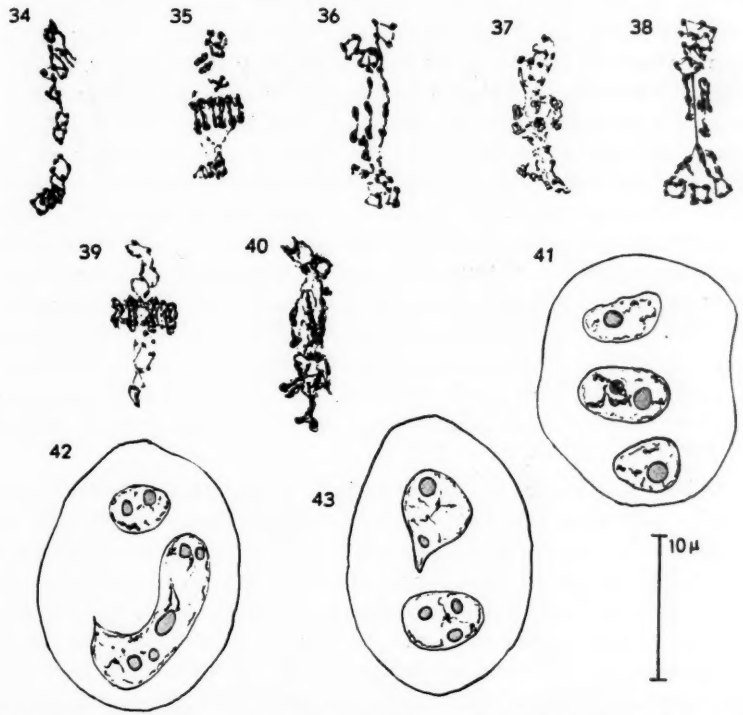
In Figs. 22 and 25 some univalents lie quite a distance from the metaphase plane and an irregular metaphase plane appears to be present. It is believed, however, that this position arose secondarily.



Figs. 19—33. *Chondrilla juncea* from Stockholm. — The first meiotic division in the PMC's of the partially asynaptic type. All PMC's from the same loculus. — 19—20, metaphases. — 21—26, anaphases; the chromatids of the univalents separate but fail to move to the poles. — 27—28, telophases. — 29—33, interphases lying successively after the telophases; 29—30, in polar view.

When the bivalents enter the anaphase the spindle undergoes a considerable stretching and probably some univalents are then passively displaced. For some reason or other a stretching of the spindle has failed in Figs. 23—24 and in these cases all the univalents are well accumulated on the metaphase plane (*cf.* also p. 308).

The fact that all univalents have divided into their two chromatids is characteristic at this stage. As in the Uppsala plants, however, the chromatids lack the capacity to pass to the poles and therefore remain on the equator. Their later fate is shown in Figs. 27—28. These two figures represent two PMC's lying next to that in Fig. 26. All chro-



Figs. 34—43. *Chondrilla juncea* from Stockholm. — The first meiotic division in the PMC's of the partially asynaptic type. — 34—40, telophases. — 41—43, interphases.

mosomes have begun to dissolve, forming a long spindle-shaped chromatin mass. As a matter of fact this dissolution has begun still earlier in the daughter chromosomes of the bivalents (*cf.* Figs. 23—26). Figs. 27 and 28 correspond to the stage of Fig. 14 in the Uppsala plants representing an early telophase. Normally two groups of telophase chromosomes are formed in each PMC (*cf.* Fig. 9). In this case, however, only one is present because of the numerous univalents and their incapacity of anaphase separation. Such elongated telophase figures are common in these plants, but in this loculus there were only these two. In Figs. 34—40 therefore some other cases from different loculi have been drawn to show how these stages can look. A characteristic feature is the elongated shape and the stickiness of the chromosomes. A true nucleus membrane is not observed in these stages (nor in Figs. 9, 11

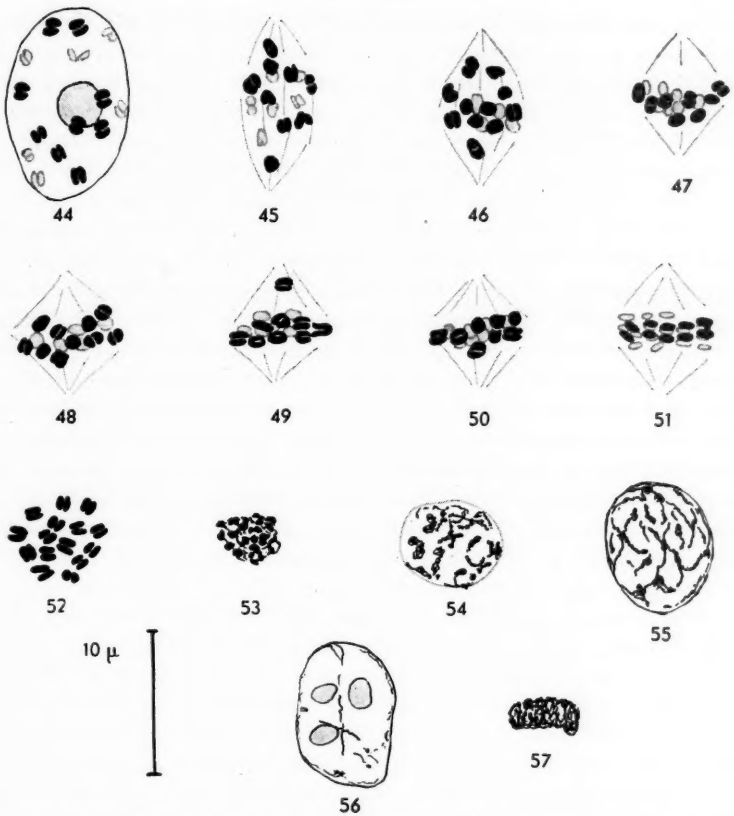
and 14). In Fig. 29, on the contrary, a thin membrane is visible and a clear one in Fig. 30. These figures represent two PMC's lying in the same loculus as that of Fig. 28 and immediately above it. Fig. 29 is in approximately the same stage as Fig. 28, though the spindle-shaped chromatin body is in this case in polar view. Perhaps this orientation makes the membrane visible. In the figure it is also seen how the chromatin body has shrunk and looks as if it had contracted from the sides. The succeeding PMC (Fig. 30) is orientated in the same manner as in Fig. 29 and has a clear membrane. It also shows how the nucleus has increased in size, the chromosomes at the same time assuming an interphase shape. The same thing holds true of the next PMC (Fig. 31), this, however, being longitudinally orientated. After this comes another couple of PMC's having about the same appearance, one of which is drawn in Fig. 32. The loculus ends at the top with the PMC's containing more rounded nuclei, thin chromatin threads and several nucleoli (*cf.* Fig. 33).

Thus, this partial asynaptic meiosis results in restitution nuclei forming unreduced interphase nuclei of the appearance in Fig. 33. In this plant it is very common to come across whole loculi with such interphases. By examining these loculi, PMC's of the same appearance as that of those in Figs. 41—43 are sometimes to be observed. These PMC's have no doubt originated from cases as in Figs. 34—40 when the formation of a restitution nucleus has failed to take place. However, such cases are unusual but are of a certain significance for the interpretation of some deviations in later stages (*cf.* p. 311).

As mentioned above, loculi with partial and total asynapsis are of about the same frequency in these plants.

Figs. 44—57 show the course of division in the totally asynaptic loculi.

Figs. 44—50 represent seven PMC's in the same loculus successively from one end of the loculus to the other. At the bottom lie diakineses and at the top metaphases. The seven selected PMC's represent the outlines of the course of division. The diakineses are of the appearance indicated in Fig. 44. All fifteen chromosomes are already longitudinally divided, which is easy to see if the staining has not been too dark. After the diakineses follow prometaphases with chromosomes scattered on the spindle (Figs. 45—46). The succeeding PMC's are characterized by a more evident accumulation of the univalents on the middle of the spindle (Figs. 47—48), and in the upper part of the loculus a meta-



Figs. 44—57. *Chondrilla juncea* from Stockholm. — The first meiotic division in the PMC's of the totally asynaptic type. 44—50 and 51—56 from two different loculi. — 44, diakinesis with fifteen longitudinally divided univalents. — 45—48, prometaphases. — 49—52, metaphases. The chromatids separate but fail to move to the poles. — 53, telophase (»contraction nucleus») originating from a metaphase plate in polar view. — 54—56, interphases lying successively. — 57, telophase (»contraction nucleus») in side view.

phase plate is formed with the univalents in a metaphase position (Figs. 49—50).

Unfortunately no loculi are to be found containing all stages from diakineses to interphases inclusive, and as a matter of fact this applies to normal sexual Composites, too. Because of that we have to study another loculus in order to get an idea of the continuation of the division

in Figs. 44—50. Figs. 51—56 represent such a loculus and the PMC's are drawn in the same order as in the preceding loculus. A row of PMC's having the appearance shown in Fig. 51 lie in the lower part of the loculus. That Fig. 51 really is a succeeding stage to Fig. 50 has been proved by studies in other loculi containing both stages at the same time. As in the previous type of division, the univalents divide into their two daughter chromatids lying parallel to the metaphase plane, as appears in Fig. 51. The row of PMC's with this appearance is completed with the PMC in Fig. 52. It is a metaphase also, but in polar view. The two immediately following PMC's have quite another appearance but, as both are of the same shape, only one of them is illustrated (Fig. 53). The chromosomes in this PMC have dissolved into a much contracted chromatin mass. On a comparison of this figure with Figs. 9, 11, 14, 27 and 28 it becomes evident that it is a case of a telophase. These two telophases, no doubt, have originated from metaphases orientated in the same way as in Fig. 52. However, such telophases in side view are common and such a stage from another loculus is drawn in Fig. 57. The two telophases (one of which is drawn in Fig. 53) are then followed by some PMC's of the appearance shown in Fig. 54. The nucleus has in this case begun to increase in size and the chromatin has assumed an interphase shape. In the succeeding PMC's (Fig. 55) the nuclei have further increased in size and the loculus ends with interphases (Fig. 56) of the same appearance as in the previous type of division (*cf.* Fig. 33).

Thus, neither in the totally asynaptic loculi nor in the partially asynaptic loculi do the univalents divide mitotically at anaphase. Although stages as in Fig. 51 suggest that anaphase is imminent, there was not a single case in which such a stage was observed. Instead, they always changed into telophases of the appearance pictured in Figs. 53 and 57 and these afterwards into interphases.

The metaphase plates of the totally asynaptic type of division are always regular, as in mitosis, though one chromosome sometimes lies to one side of the plate (Fig. 49). However, the irregular appearance of the corresponding stage of the partially asynaptic type of division (*cf.* Figs. 22 and 25) never occurs in this case. The irregularities of the previous type of division were considered to be secondary, caused by the stretching of the spindle during the anaphase of the bivalents (*cf.* pp. 303—04). This interpretation is verified by the facts in the totally asynaptic type of division. As a matter of fact, a stretching of the spindle does not occur and the univalents remain well accumulated in the metaphase plane. Hence, the stretching of the spindle should depend

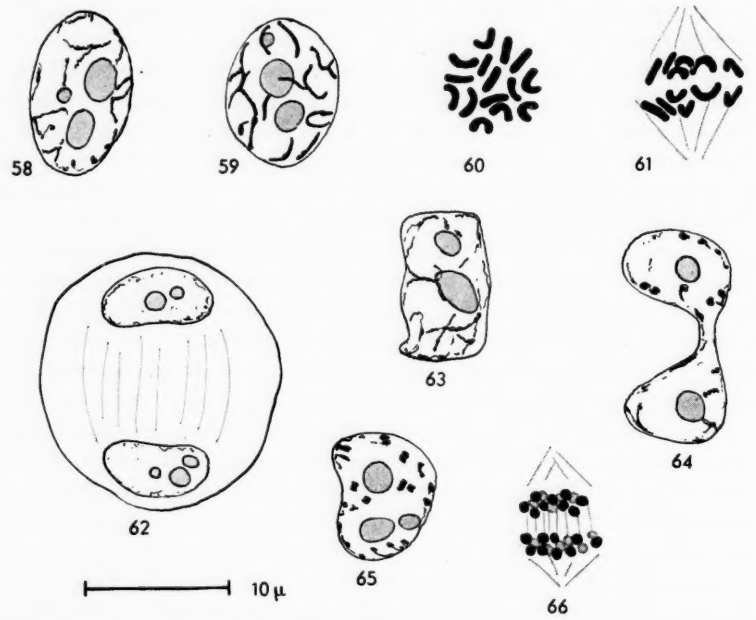
on the presence of bivalents. If the bivalent formation fails, the stretching also fails.

Thus, from the foregoing it appears that the totally asynaptic type of division is completed with a restitution nucleus though of a more rounded shape. It is formed in the metaphase stage, one might say, whereas that of the partial type is formed in the anaphase stage. This is, however, not quite right. Referring to the previous type of division, the univalents were at metaphase when the bivalents were at anaphase or even in a later stage. Hence, the univalents enter metaphase later than the bivalents. No doubt this delay occurs in the totally asynaptic type, too. As no bivalents occur in this, one cannot, it is true, verify the matter, but very probably the PMC's in, *e. g.*, Figs. 51—52 correspond in time to the anaphase of a normal meiosis. This is confirmed by the fact that the prometaphase seems to be of long duration (which also applies to the prometaphase of the *univalents* in the previous division). When only the univalents are taken into consideration, the restitution nucleus in either type may therefore be said to be formed during metaphase, whereas, when the time relations of the normal meiosis are taken into consideration, it is formed during anaphase.

In this way the two types of division proceed in the first meiotic division of the Stockholm plants, different in form but very similar in essentials. Deviations were never observed, but they seem to predominate in all loculi.

The first meiotic division is regularly followed by the second one and a close examination of it has also been made. Unlike the first one it is, as a matter of fact, nearly always normal and is illustrated in Figs. 58—62. The PMC's in Figs. 58—61 originate from the same loculus and lie successively. The loculus begins with interphases of the appearance shown in Fig. 58. Fig. 59 represents the prophase, Fig. 60 the metaphase, and Fig. 61 the anaphase (all chromosomes are not drawn). Later some further anaphases follow and are of about the same appearance. The chromosomes are, it appears, more mitotical in this division, which is rather common among the Composites. Fig. 62 shows the result of the division.

But for two remarkable exceptions the second meiotic division proceeds in this way in all cases observed by the author. In two different loculi, however, it was found that the division proceeded in a way shown in Figs. 63—66. They exhibit four PMC's lying successively in the same loculus. Figs. 63—64 are interphases. Evidently the nucleus in Fig. 64 has retained its shape from the preceding division, which was



Figs. 58—66. *Chondrilla juncea* from Stockholm. — The second meiotic division in the PMC's. 58—61 and 63—66 from two different loculi. — 58, interphase. — 59, prophase. — 60, metaphase with fifteen mitotisized chromosomes. — 61, anaphase in side view; all chromosomes are not drawn. — 62, dyad. — 63—64, interphases. — 65, prophase. — 66, anaphase with spherically contracted chromosomes.

probably of about the same shape as that in Fig. 38, for example. The following PMC (Fig. 65) represents a stage just prior to metaphase and it can already be seen that the chromatin threads are very much contracted. This appears most clearly in comparison with Fig. 59, which is a corresponding stage of the normal division. The next PMC (Fig. 66) is at anaphase and has very much contracted and almost spherical chromosomes. A comparison of this figure with Fig. 61 reveals a striking difference. After the PMC in Fig. 66 a few others follow of about the same appearance.

One more anaphase with spherically contracted chromosomes has been observed, this time, however, in a loculus with the second meiotic division proceeding normally with mitotisized chromosomes. Comparing several metaphases of the appearance of those in Fig. 60, small variations

in the degree of contraction are sometimes to be observed. Such cases as in Fig. 66, however, are very rare and difficult to explain.

In this plant the result of the two meiotic divisions is nearly always dyads. Loculus after loculus with only dyads is very characteristic. Later the dyads give rise to pollen grains of normal appearance though somewhat larger than those of the Uppsala plants. Sometimes, however, one can come across tetrads or polyads among the dyads. By reference to Figs. 41—43 these deviations may be easily explained.

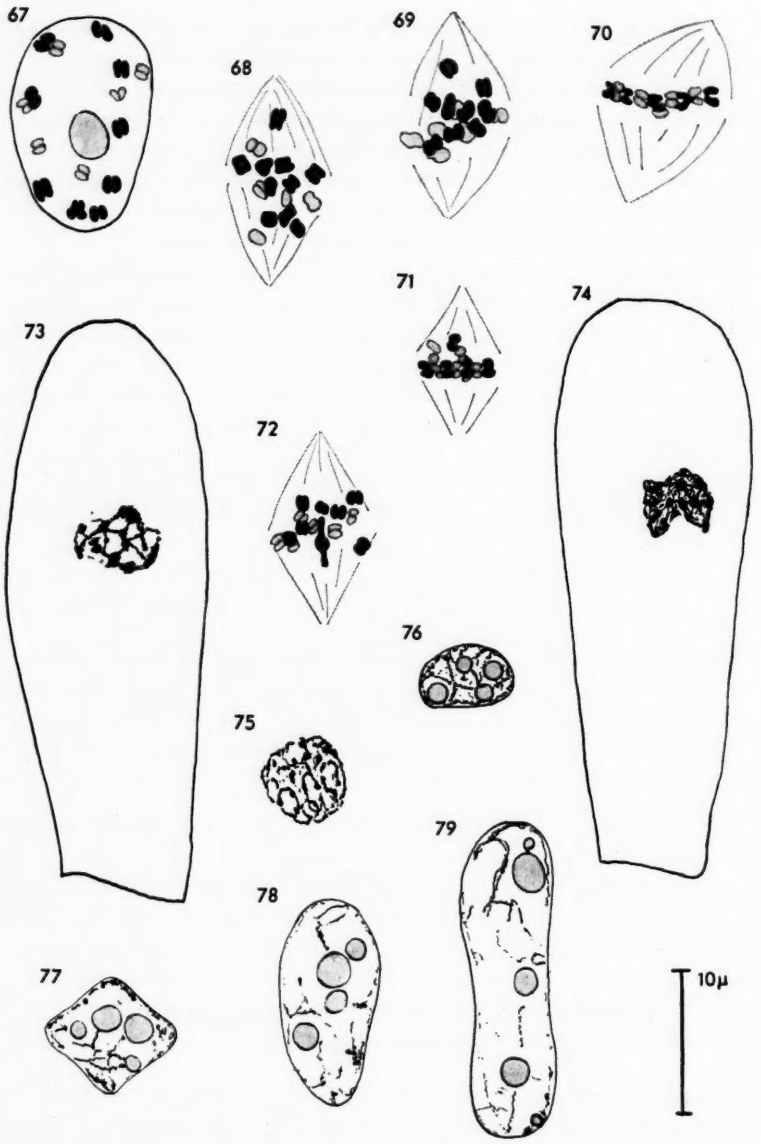
Summing up the observations made on the microsporogenesis of the Stockholm plants, the following is to be noted: Meiosis is either partially asynaptic (at most three bivalents) or totally asynaptic. In either case the univalents have the capacity of moving towards the middle of the spindle, forming there a metaphase plate. The univalents enter metaphase later than the bivalents. An anaphase separation of the chromatids of the univalents, as in mitosis, fails to appear and therefore in either case a restitution nucleus is formed, giving rise to unreduced interphases. The second meiotic division is normal and is completed with dyads.

Comparing the observations from the Uppsala (pp. 302—03) and the Stockholm plants, there is first a quantitative difference with regard to the univalents, the Stockholm plants having a much greater number. A qualitative difference is also to be noticed. In the last-mentioned plants the univalents accumulate at the equatorial plane, forming a metaphase plate, a capacity which is lacking in the Uppsala plants. In addition there are differences in the frequency of restitution nuclei. In the Uppsala plants they were rare in the first meiotic division, in the Stockholm plants, on the other hand, they occur in nearly 100 per cent of the PMC's.

On account of these differences I consider that the plants belong to two different clones, though other opinions on this matter may be asserted (*cf.* p. 318).

III. MEIOSIS IN THE EMBRYO-SAC MOTHER-CELLS (EMC's).

As already mentioned, it is much more difficult to study the divisions in the EMC than in the PMC of the Composites. To this rule *Chondrilla juncea* is no exception. In studying the EMC's of this plant all division stages observed have, however, shown such great similarities



Figs. 67—79. *Chondrilla juncea* from Stockholm. — The first meiotic division in the EMC's. — 67, diakinesis with fifteen longitudinally divided chromosomes. — 68—69,

to the conditions in the PMC's of the Stockholm plants that it has not been difficult to reconstruct the course of the division.

In studying the EMC's attention was focused on the Stockholm plants and all figures submitted in the following description are taken from them. Stages from the Uppsala plants have shown the same type of division to be present in them, too, in spite of the great difference in the microsporogenesis.

In the following account the Stockholm plants are always referred to when comparisons are made with the microsporogenesis.

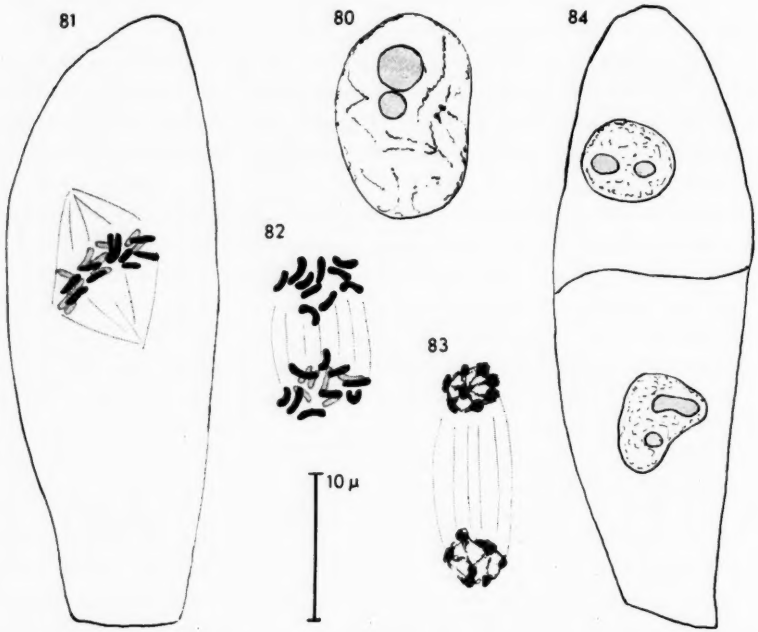
Figs. 67—84 show the outlines of the macrosporogenesis.

The prophase stages seem to be normal, but a pairing of the chromatin threads has not been observed. The following stages most closely correspond with the totally asynaptic type on the male side (Figs. 44—57). The diakinesis in Fig. 67 having fifteen longitudinally divided chromosomes is identical with that in Fig. 44. The following four figures (68—71) show the univalents lying scattered on the spindle at prometaphase, later, however, forming a metaphase plate in the same manner as in Figs. 45—52. (In Figs. 70—71 all chromosomes are not drawn.) Only a single case of bivalent formation has been observed and this is drawn in Fig. 72. It was a case of a single bivalent and from the figure it appears that on the female side, too, the univalents evidently enter metaphase later than the bivalents.

The metaphase in Fig. 70 gives the impression that the anaphase is imminent. However, as on the male side, such stages have never been observed. Instead of the anaphase there were six cases such as in Figs. 73—74. They correspond most closely with Figs. 53 and 57 and are to be considered as telophases. It is therefore evident that the metaphases here, too, as in the microsporogenesis, never change into anaphases but are dissolved and form telophases. Later, the telophase nuclei gradually increase in size and change into interphases with several nucleoli (Figs. 75—80) in the same manner as in Figs. 53—56. Frequently the interphase nucleus assumes a very elongated shape as in Fig. 79, probably in some way depending on the shape of the cell.

Thus, the first meiotic division is completed with a restitution nucleus having the limited and rounded shape typical of the totally asynaptic division. The fact is that all the six telophases observed were

prometaphases. — 70—71, metaphases; the chromatids separate but fail to move to the poles. — 72, one bivalent in metaphase; the univalents enter metaphase later than the bivalents. — 73—74, telophases (»contraction nuclei»). — 75—79, interphase nuclei successively increasing in size.



Figs. 80—84. *Chondrilla juncea* from Stockholm. — The second meiotic division in the EMC's. — 80, interphase. — 81, metaphase. — 82, anaphase. — 83, telophase. — 84, dyad. All chromosomes are not drawn in 81 and 82.

of about the same appearance as in Figs. 73—74. No single case with elongated telophases, so common in the partially asynaptic meiosis (*cf.* Figs. 27—28 and 34—40), has been observed. As in addition only one EMC has shown bivalent formation, it might be concluded that the totally asynaptic type of division is predominant in the EMC's. On the other hand, it stands to reason that the number of division stages in the EMC's compared with that in the PMC's will be small and therefore the whole may be a result of chance. However, the essential point is that the present investigation has shown that total asynapsis as well as partial asynapsis occurs, and in an extensive exmination one *ought* also to come across elongated telophases with sticky chromosomes. Fig. 72 argues in favour of this being the case.

The second meiotic division is quite normal. Fig. 81 represents a metaphase and, like the anaphase in Fig. 82, shows very much

mitotisized chromosomes. In Fig. 81 all chromosomes are not drawn, and in Fig. 82 five chromosomes are missing in the upper part, as this was cut by the knife. Fig. 83 shows a telophase (*cf.* Figs. 73—74) and Fig. 84 is the completed dyad. In all cases studied only normal dyads have been observed. Later, the chalazal dyad cell forms a normally organized eight-nucleated embryo-sac.

Summing up the observations on the EMC's, it is evident that in all its essential parts meiosis corresponds with the events in the PMC's of the Stockholm plants. The totally asynaptic type appears to be predominant.

IV. DISCUSSION.

The present investigation has shown that the pseudohomotypic division (GUSTAFSSON, 1935, and later) does not occur in *Chondrilla juncea*. Instead, this plant forms its unreduced embryo-sacs by restitution nuclei. In this respect *Chondrilla* corresponds in all essentials with *Taraxacum*, which was recently investigated by FAGERLIND (1947 c) and BATTAGLIA (1948). The order in which they place the division stages of the EMC is, no doubt, right. Should, however, any uncertainty still exist, this must quite disappear when comparison is made with the conditions in *Chondrilla*. In this plant the totally asynaptic division (Figs. 44—57) is practically identical with that in the EMC's of *Taraxacum*. The division of *Chondrilla*, however, occurs not only in the EMC's but in the PMC's, too, where hardly any doubt can exist about the succession of the division stages.

Parallel with *Taraxacum*, FAGERLIND has also studied the formation of restitution nuclei in *Rudbeckia* (1946), *Erigeron* (1947 b), and *Hieracium* (1947 a). In doing this he has been able to confirm the earlier observations of ROSENBERG (1926—27) that restitution nuclei (contraction nuclei) can be formed in all stages of meiosis from the prophase to the anaphase. In *Chondrilla*, on the contrary, so far as the author has been able to discover, they are only formed in the metaphase or the anaphase stage. Now, however, one must be clear about the meaning of these stages, as pointed out above. As a matter of fact, in *Chondrilla* the univalents enter the metaphase in a stage most nearly corresponding to the anaphase of the normal meiosis. This probably applies to *Taraxacum*, too, as appears from GUSTAFSSON's (1934) and FAGERLIND's (1947 c) observations that the prometaphase is prolongated. Hence, as in *Chondrilla*, the metaphase of the univalents must be delayed. The restitution (contraction) nucleus later formed from this metaphase is

nothing but the early telophase. The relatively great number of univalent metaphases to be seen in certain loculi points to the fact that this stage is also prolongated (*cf.* Figs. 21—26 where, however, all PMC's in the loculus are not drawn). There is therefore no reason to believe that the telophase is abnormally early, at least not in *Chondrilla*. More likely the univalent metaphase (that is the anaphase of the normal meiosis; *cf.* Figs. 21—26) changes into the telophase at a normal point of time. In the meiosis of *Chondrilla* the only deviation from the normal time scheme is a delay of the univalent metaphase, and when the metaphase is completed it lasts through to the telophase.

A pronounced contraction stage is characteristic of these restitution nuclei in *Chondrilla*, *Taraxacum*, *Erigeron*, and *Rudbeckia* and has led to their being called contraction nuclei. These nuclei, however, are formed in the telophase stage, and as already pointed out by FAGERLIND (1947 a) a strong contraction of the chromatin mass is normal and typical at this stage. Hence, there is no particular reason to give them the special name of contraction nuclei, and therefore, like FAGERLIND, the author considers that they should be called restitution nuclei.

The present investigation of *Chondrilla* also throws light upon the conditions of *Erigeron cfr. annuus*. This plant was already studied by the author on an earlier occasion (1944), who at that time believed he could establish the occurrence of four different types of divisions in the EMC: one pseudohomotypic, one meiotic, one mitotic, and one sticky. Moreover, he came across several EMC's with contracted nuclei which were not possible to explain. It was among other things these nuclei that caused FAGERLIND (1947 b) to make an examination of *Erigeron*. On that occasion he was able to show that on the whole a pseudohomotypic division does not occur in this plant. Instead, it formed its unreduced embryo-sacs by a metaphase restitution nucleus in the same manner as he has demonstrated for *Taraxacum*. The contracted nuclei fitted very well into the course of division in *Erigeron*. On the other hand, the sticky metaphases—anaphases with long slender chromosomes which are drawn in Fig. 1 *e—g* (BERGMAN, 1944) were more difficult to explain. FAGERLIND (1947 b) himself has come across three such cases and considers them to be prometaphases and indicative of restitution nuclei being formed during the prometaphase. Following these studies in *Chondrilla*, however, another interpretation may be presented as well. These figures with long slender sticky chromosomes closely resemble those which are drawn in the partially asynaptic type of division occurring in the male organ of *Chondrilla* (*cf.* Figs. 34—40).

Nearly always these resulted in elongated restitution nuclei and such were also observed in *Erigeron* (*cf.* BERGMAN, 1944, Fig. 1 *h—i*). These elongated sticky chromosome figures in *Chondrilla*, however, always implied the presence of bivalent formation. As seen in Fig. 1 *m—l* (BERGMAN, 1944), such nuclei sometimes occur in *Erigeron* also. It is the opinion of the author that these facts may be now interpreted in the following way: the unreduced embryo-sacs of *Erigeron cfr. annuus* are formed in two different ways, (1) by a totally asynaptic meiosis resulting in more rounded restitution nuclei of the appearance in Figs. 44—57, (2) by a partially asynaptic meiosis resulting in elongated restitution nuclei of the appearance in Figs. 19—40.

Thus, *Chondrilla juncea* and *Erigeron cfr. annuus* seem to form their embryo-sacs in about the same manner. The only difference is that in *Chondrilla* the totally asynaptic type seems to dominate while in *Erigeron* the total and the partial types seem to be more evenly distributed among the EMC's.

The mitotic type of division which (in rare cases) was considered to be present in *Erigeron* is based on Fig. 1 *k* in a previous paper (BERGMAN, 1944). However, with regard to the conditions in *Chondrilla* this figure probably represents the metaphase of the second meiotic division. Comparing it with Figs. 18, 60—61 or 81 in the present paper, we find that the similarities are very great.

The four different types of division which in a previous paper (BERGMAN, 1944) were considered to be present in the EMC's of *Erigeron cfr. annuus* may therefore now be reduced to two types into which all stages observed can be fitted.

In other respects, too, *Chondrilla* and *Erigeron* invite to comparisons. *Erigeron cfr. annuus* has the chromosome number of $2n = 27$. HOLMGREN (1919) states that the bivalents of the microsporogenesis vary from three to five and that the rest of the chromosomes are univalents. In spite of this, normal tetrads are nearly always formed (p. 19). The individual of the same species examined by the present author showed quite another microsporogenesis (BERGMAN, 1944). Generally the bivalents were 10—11 and in some cases trivalents were observed. As a rule, the tetrads were abnormal with micronuclei. Micro pollen grains were also usual.

With regard to the microsporogenesis *Erigeron cfr. annuus* may evidently be divided into two different forms, highly resembling the conditions of *Chondrilla juncea*. Here, too, we have individuals differing with regard to their microsporogenesis. Above, it was considered

that they belong to two different clones and therefore it was asserted that these differences are genetically conditioned. In spite of the parthenogenetical reproduction, the capacity of segregation in this type of apomixis is still present, as has been pointed out earlier (*cf.* BERGMAN, 1941, pp. 33—34). However, GUSTAFSSON and NYGREN (1946) have shown that the meiosis in the anthers of *Hieracium robustum* is affected by changes of temperature. It might then be thought that these differences in the microsporogenesis of *Chondrilla* and *Erigeron* are a result of environmental factors. Against this, however, the following can be said: firstly, it is unknown to what extent GUSTAFSSON and NYGREN's results may be generalized, secondly, the differences in environmental conditions must be quite small in the case of *Chondrilla*. Uppsala and Stockholm are not far from each other and the collections were made on Sept. 6 and on Sept. 7, 1949, respectively. Naturally this problem is only to be solved by experimental methods, and the writer hopes to be able to carry out such on a later occasion. However, as the facts at present argue in favour of genetical differences, the clone theory has been adopted by the author.

This investigation was made at the Institute of Systematic Botany of the University of Uppsala, and I am very much indebted to Professor J. A. NANNFELDT for his kindness in giving me working space at his institute.

SUMMARY.

(1) Plants of *Chondrilla juncea* L. from the botanical gardens of Uppsala and Stockholm have been examined with regard to their sporogenesis.

(2) The Uppsala and Stockholm plants have the same macrosporogenesis; they differ, however, considerably in regard to their microsporogenesis. Both are triploid and have a chromosome number of $2n = 15$.

(3) The Uppsala plants have most commonly the association of $6_{II} + 3_{I}$ in the PMC's. Univalents fail to move and to assemble on the equatorial plane. The result of the microsporogenesis is nearly always normal tetrads.

(4) The Stockholm plants have a totally asynaptic meiosis in about half the number of the loculi of the anthers; in the remaining loculi a partially asynaptic meiosis with at most three bivalents occurs. All the

univalents have the capacity of arranging themselves on the equatorial plane, forming there a metaphase plate. The anaphase separation of the daughter chromatids of the univalents, however, fails and, instead, restitution nuclei are formed. The result of the microsporogenesis is almost always dyads.

(5) The stretching of the spindle in the first meiotic division depends on the presence of bivalents. If the bivalent formation fails, the stretching also fails.

(6) The macrosporogenesis in both types of plants corresponds to the microsporogenesis of the Stockholm plants. The totally asynaptic type of division seems to predominate in the EMC's.

(7) Comparisons between *Chondrilla*, *Taraxacum* and *Erigeron* have been made and differences and similarities have been discussed. The cause of the differences in the microsporogenesis of different plants of *Chondrilla juncea* has been discussed and the author suggests that the plants may be regarded as belonging to different clones.

Since this paper was completed BATTAGLIA has published an investigation on *Chondrilla juncea* (BATTAGLIA, E. 1949. L'alterazione della meiosi nella riproduzione apomittica di *Chondrilla juncea* L. — Caryologia, Vol. II, N. 1, pp. 23—30). He deals only with the development of the embryo-sac, however, and the critical stages of the first meiotic division drawn by him are relatively few and in no way contradict the present author's results.

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STUDIES ON MUTATIONS IN BARLEY

I. SUPERDOMINANT FACTORS FOR INTERNODE LENGTH

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INTRODUCTORY REMARKS.

IN his classical work of 1866 MENDEL describes as dominance the kind of interaction between two alleles when, in a hybrid, the phenotypical manifestation of the one allele so completely covers that of the other allele »dass es schwierig oder ganz unmöglich ist das andere an der Hybride aufzufinden». However, later experience was to show that this case, complete dominance, was relatively seldom to be met with, the dominance usually being incomplete. Gradually the strict distinction of MENDEL was replaced by less extreme definitions of the dominance phenomenon, especially for the convenient description of mutant genes in *Drosophila*. Thus, the Nomenclature Committee of the American Society of Naturalists says in its report (LITTLE, 1921), on which later international agreements are based: »Factors may be considered dominant which produce an easily recognized departure from type, when heterozygous».

When the phenotypical expression of a heterozygote lies in the region between both homozygotes ($AA > Aa > aa$) we speak about partial or imperfect dominance, and only when the heterozygote cannot be distinguished from one of the homozygotes ($Aa = AA$) have we complete dominance. As already mentioned, this is not a common case, but it need not be the extreme which cannot be passed. FISHER (1918) mentions the possibility of what he calls *superdominance*, and HULL (1946, 1949) uses the term overdominance or overcomplete dominance for the same thing, namely to denote the case when the phenotypical expression of the heterozygote falls outside the region between both homozygotes ($Aa > AA$). Thus, superdominance may appear as a kind of monohybrid heterosis affecting a certain character. Later, FISHER (1930, 1932) reckons with heterozygotes having a »selective advantage over both homozygotes», and HULL (1946), CROW (1948) and others postulate the existence of superdominant viability factors for a full

understanding of the phenomena of hybrid vigour and inbreeding degeneration.

A CASE OF SUPERDOMINANCE IN BARLEY.

During his cytogenetical studies of spontaneous and induced mutations in barley (*Hordeum distichum*) the present author has obtained some results which may be taken as a rather clear case of superdominance. Even if the empirical basis, *i. e.* the number of plants analysed, is not very large and certain objections may be raised against the experimental conditions, the results appear so interesting that I think they might be worth a close consideration.

The results refer to diallelic crossings between three x-ray induced mutations of the type denoted as *erectoides* mutations, and with their mother line, Bonus barley. (*Cf.* GUSTAFSSON and MAC KEY, 1948). The mother strain is a high-yielding two-row variety of the *nutans* type. The mutations, which are characterized mainly by a more or less reduced culm and rachis internode length, are all allelic, *i. e.* they give an erectoid F_1 when crossed with each other.

The material is presented in Table 1. The lengths of culm and head are determined on the longest shoot. The seed fertility is obtained by counting the numbers of fertile and sterile florets on the whole plants. As appears from the table, the mutations are all distinguished from each

TABLE 1.

	Number of plants	Heads per plant	Length of culm cm	Length of head mm	% seed-fertility
Bonus	15	7,0	75,9	110,1	96,2
Erectoides 19	13	7,2	65,5	75,5	93,6
Erectoides 21	14	8,3	74,1	86,0	95,8
Erectoides 23	14	7,7	69,9	76,5	95,9
Er. 19 × Bonus	9	8,5	81,7	119,8	95,8
Bonus × Er. 19	14	8,7	79,8	118,9	95,5
Er. 21 × Bonus	13	8,2	83,3	124,3	69,4
Bonus × Er. 21	8	7,0	78,6	124,0	70,7
Er. 23 × Bonus	16	8,8	80,9	121,9	94,9
Bonus × Er. 23	8	6,0	78,0	107,5	95,5
Er. 21 × Er. 19	14	7,7	74,4	79,8	70,6
Er. 19 × Er. 21	16	7,5	74,0	83,3	73,0
Er. 21 × Er. 23	7	8,0	73,0	86,0	67,8
Er. 23 × Er. 21	18	8,6	75,1	87,4	70,5
Er. 19 × Er. 23	2	10,0	77,0	91,0	93,2
Er. 23 × Er. 19	17	8,2	73,7	83,5	95,2

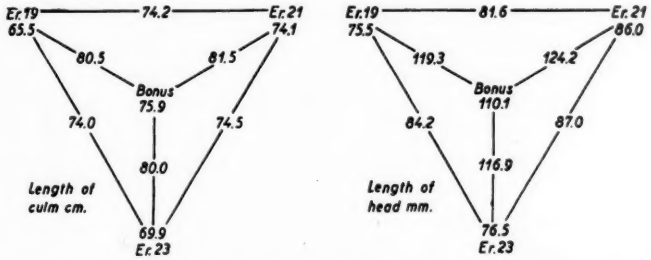

Fig. 1. Length of culm and head in the four cross parents and their hybrid combinations.

other as also from the mother line with respect to length of culm and head. The four cross parents and their six F_1 combinations are compared in Fig. 1. Here we immediately notice the interesting phenomenon that in all combinations, except *Er.* 19 × 21, the heterozygotes have longer culms and longer heads than both homozygotes. In all combinations with Bonus, as also between *Er.* 19 and 23, the differences are very conspicuous. Unfortunately the material is rather small for a statistical analysis, but the figures nevertheless look convincing.

It must be admitted that certain objections may be raised against the experimental conditions. No special precautions were taken to ensure every plant identical conditions in the field. Owing to a slightly lower germination percentage of the F_1 seeds, and also because the F_1 rows were not always filled out with plants, some of the F_1 plants possibly happened to become a little better spaced than the parent plants. The distances between rows and between sown seeds were the same for both F_1's and parents. From Table 1 it appears that on an average the F_1 plants have a somewhat higher number of heads per plant than the parent lines. As it is known that a thin stand gives plants with more tillers and longer culms and heads, we may here suspect a source of error. To a certain degree we can eliminate this error by grouping the material into different classes with respect to number of heads and then by comparing plants having a similar number of heads per plant. This has been done for the three combinations with the mother line in Table 2. As can be seen, the heterozygotes lie above Bonus in 15 of the 18 classes. The average difference F_1—Bonus is, concerning length of culm, 3,12 cm ± 0,81, *i. e.* a significant difference, $0{,}01 > p > 0{,}001$. For the length of head the mean difference is proportionally higher, 6,11 mm ± 2,85, but not so significant owing to an

TABLE 2.

Number of heads per plant	Length of culm			Length of head			Number of plants		
	Bonus	Er. 19	F_1	Bonus	Er. 19	F_1	Bon.	Er.	F_1
1—5	70,8	58,0	73,0	102,8	65,0	97,5	5	3	2
6—8	77,0	65,6	80,0	110,4	77,2	117,8	2	5	7
9—10	78,8	70,0	81,8	114,0	80,2	122,9	8	5	14
	Bonus	Er. 21	F_1	Bonus	Er. 21	F_1	Bon.	Er.	F_1
1—5	70,8	70,0	72,5	102,8	71,0	111,5	5	1	4
6—8	77,0	71,6	81,4	110,4	84,8	123,6	2	5	5
9—10	78,8	76,3	84,6	114,0	88,7	127,0	8	8	12
	Bonus	Er. 23	F_1	Bonus	Er. 23	F_1	Bon.	Er.	F_1
1—5	70,8	73,5	76,9	102,8	79,5	115,0	5	2	6
6—8	77,0	65,8	75,0	110,4	73,2	99,0	2	8	4
9—10	78,8	72,5	82,7	114,0	81,8	123,3	8	4	4

increased variation, $0{,}05 > p > 0{,}02$. Without overstraining the data it may be said that there seem to be clear tendencies to superdominance in the material. Repeated crossings will give conclusive information.

The somewhat peculiar fertility figures given in Table 1 ought here to be discussed. As appears from the table, the seed fertility of the parents is about 95 % and the same is also true for the hybrid combinations in which *Erectoides* 21 does not take part. *Er.* 21 gives in all its three combinations a marked decrease in fertility to about 70 %. This suggests the occurrence of some chromosomal rearrangement, which was also confirmed by the cytological analysis. The *Er.* 21 line used proved to be homozygous for a reciprocal translocation, giving a ring of four chromosomes at meiosis in the heterozygotes. The translocation does not markedly influence the manifestation of the superdominance. Data from the F_2 show that it is inherited independently of the erectoides factor. The following frequencies were obtained in the F_2:

fertile nutans	:	semisterile nutans	:	fertile erectoides	:	semisterile erectoides
128	:	143	:	42	:	41

A χ^2 analysis of these observed frequencies, using the formulae of LAMM (1948), gave:

Segregation: fertile—semisterile $\chi^2 = 0{,}55$, p close to 0,5
» : nutans—erectoides $\chi^2 = 0{,}46$, » » » »
Independent inheritance $\chi^2 = 0{,}31$, » » » »

There are also several other cases known in barley in which morphological mutations have been induced together with segmental interchanges, either closely linked with or independent of them (HAGBERG and the present author, unpublished).

DISCUSSION.

The concept of *superdominance*, seen in relation to the heterotic phenomena, may under certain conditions be synonymous with monohybrid heterosis, *i. e.* heterosis due to heterozygosity for a single factor. However, considering the actual confusion in the meaning and use of the term heterosis (*cf.* SHULL, 1948), I think that superdominance is a simpler and more generally applicable term and also a logical and useful extension of the dominance concept. Among other things, SHULL (1948) criticizes the expression »negative heterosis». It should be noted that superdominance may manifest itself in a positive as well as in a negative direction. The term is also applicable to such characters as those for which it would be less adequate to use the term »heterosis» *sensu* SHULL (»The greater vigor or capacity for growth frequently displayed by crossbred animals or plants . . .»).

I think, however, that it might be advantageous to delimit the use of the term *superdominance* only to single gene relations, just as WIGAN (1944) and MATHER (1946) also want to restrict the use of *dominance* to the interaction within a single pair of alleles. For the phenotypical effect of polygenic combinations WIGAN proposed the term *potence*.

Heterosis should, accordingly, be used preferably for a positive effect when the genetical basis is known to be polyfactorial, but it may also be employed when the factorial basis is not known or, for other reasons, is not considered.

Another use for the term heterosis, *monohybrid heterosis*, would be to denote an increased phenotypical effect in one or several characters, due to heterozygosity in two alleles which in themselves do not affect any of these characters (which in most cases means that they do not deviate from each other in their effects on the characters in question).

I am not going to discuss in detail what characters may be ranged under the term heterosis. SHULL (1948) seems to mean vegetative vigour when he speaks about heterosis. DOBZHANSKY (*e. g.* 1949) defines heterosis as an »evolutionary adaptation». An increased vegetative vigour should, according to him, not be called heterosis if it does not imply an increased competitive ability. I think it would be best to let

heterosis be applicable to all kinds of characters, whether they have a selective value or not, if only it is specified in every case what character is concerned.

Concerning the literature on monohybrid heterosis I want to refer to GUSTAFSSON (1946, 1947; *cf.* also GUSTAFSSON and MAC KEY, 1948) who in addition to his own results also discusses several earlier cases of monohybrid heterosis. (*Cf.* also JONES, 1945.) Most of these cases deal with genes which lead to lethality or at least highly reduced viability when homozygous, but which in heterozygous condition give »heterosis» as compared with the normal homozygotes. The heterotic effects in these cases refer to such characters as earliness, vegetative development and reproduction, size of plants and their various parts, number and size of the seeds, preservation of seed germination, *etc.* in plants and increased competitive value in juvenile stages in some animals.

All except one of these cases deal with heterosis concerning characters which cannot be said to belong to the true field of action of the effective pair of alleles. It would, thus, be illogical to speak of superdominance in these cases.

The exception mentioned is the *Antirrhinum* case of STUBBE and PIRSCHLE (1940). Here a chlorophyll factor, reducing viability when homozygous, produces heterosis in respect of several of the above-mentioned characters in heterozygous condition. The heterotic effects are summarized by the authors as »einen starken über das Normale hinausgehenden Leistungszuwachs». Most interesting, however, is the observation that the chlorophyll content, which in the recessive homozygote is only 70—80 % of that of the normal green homozygote, in the heterozygote lies about 20 % above the normal. Considering this very character, the chlorophyll content, it seems to me justifiable to speak about superdominance rather than heterosis.

A case of possible superdominance is presented by H. and O. TEDIN (1926). This case is of special interest in this connexion, as it concerns a factor for length of culm in barley, just as in the cases reported in the present paper. The authors say: »the *Zz*-plants . . . are higher than either homozygote . . .», and further: »thus in this case the 'stimulating' effect of heterozygosity can be demonstrated in a single pair of factors». Unfortunately, the data refer to F_3 families segregating also for other factors, so it cannot be stated definitely »whether this effect shall be explained as a specific effect of heterozygosity or by linkage between *Z* and *z* and minor factors for plant height». If the first alternative is

the true one, we have here a clear case of superdominance, one of the very first detected.

Concerning the evolutionary significance of monohybrid heterosis and superdominance a few words have already been said in the introduction. In addition to the authors quoted there I want, in this connexion, also to refer to STUBBE and PIRSCHLE (1940) as well as to GUSTAFSSON (1946). It is worth mentioning that in most earlier cases of monohybrid heterosis the effect has been due to heterozygosity for deleterious or degenerative factors, whereas in the present case (as also in that of the TEDINS) the effect is due to heterozygosity for factors which in themselves show little or no difference with regard to viability. Whether the heterozygosity effects in these barley cases also affect other characters than the length of culm and head, *i. e.* whether they bring about typical heterosis, will be found out from the later analysis.

As to the possible mechanism of superdominance there is not much that can be said. As previously mentioned, the three erectoides mutations are considered allelic because they give an erectoid F_1 phenotype. If not allelic they should give a nutans F_1 with longer culm and laxer head. Now, the tendencies towards a longer culm and a laxer head, here called superdominance, could obviously be taken to indicate a partially non-allelic action of the various factors. The same non-allelic action could also account for the superdominance together with the mother strain. CROW (1948) also proposes a similar non-allelic action, *viz.* »that in the hybrid alleles might be acting on different substrates or transforming the same substrate into different products. Such alleles would be behaving as neomorphs or antimorphs and could result in the heterozygote being more extreme than either homozygote». The phenomena of dominance and heterosis are far from being fully understood, and a fertile discussion of them requires several other and also imperfectly known genetical elements to be taken into consideration, especially the nature of the gene, of gene action and of gene interaction.

SUMMARY.

Three allelic mutations reducing length of culm and head in barley are shown to give F_1 plants with longer culms and heads than either parent when crossed with each other or with the mother line. This is interpreted as a case of superdominance, the mother line being superdominant over the mutants.

The literature on monohybrid heterosis and superdominance is

briefly reviewed and a few viewpoints on the meaning and use of these terms are given.

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Communications from the Swedish Seed Association. No. 28.

STUDIES IN TRIPLOID RABBITS PRODUCED BY COLCHICINE

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IN a previous paper (1950) we have given a report of a method for experimental induction of polyploidy in animals by colchicine. Rabbits were used as experimental animals and the method applied could theoretically be expected to give triploid forms.

THE MATERIAL.

In such an experiment there were born 7 young after a normal pregnancy of 33 days. One of the young died after a weak and another on the following day. They were obviously feebly developed. One young rabbit of normal size lived for 13 days and seemed to be developing normally when it suddenly died. No cause of death could be discovered at post mortem examination. Another young one, showing quite normal development, lived for 8 weeks. Nor in this case could any cause of death be shown.

The remaining three young lived for nearly a year. One of them, a male, showed normal development and did not in any way differ from common diploid rabbits. The other two, a female and a male, showed a very rapid development. On account of the size of the erythrocytes and the sperms of the male, we considered them to be triploids.

THE SEXUAL FUNCTIONS OF THE TRIPLOID ANIMALS.

THE MALE.

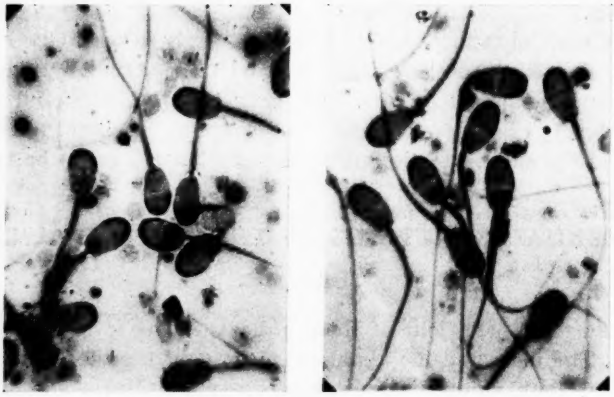
The sexual behaviour of these animals seems to be of particular interest. The male and the female were brought together at the age of 16 weeks. Both showed unmistakable sexual instincts and the male tried several times to mount the female, though this was prevented. When brought together with normal females later, the male behaved in the same manner. However, his libido was not very strong. If the

female was averse, he soon got tired of mounting her and seated himself quietly in a corner without paying her any more attention.

At the age of 23 weeks the male was allowed to mate with a normal female in heat, but no detectable pregnancy resulted, nor were any young born. The experiment was repeated at the age of 30 weeks with another female in heat, though with as little success as before. These females had previously been mated with normal males and both had given birth to normal young. After these experiments both the females continued to be fertile with normal males.

At the age of 33 weeks the male was again allowed to mate with a normal female in heat. After normal pregnancy five normally developed young ones were born. Three of them, however, died immediately after birth and the remaining two in the following twenty-four hours. No cause of death could be demonstrated. There were females as well as males. The testes showed a development adequate for new-born male rabbits but the ovaries contained only very few true oogoniums and oocytes.

Some days later the triploid male again mated a female in heat. After 31 days this female gave birth to 8 young, one of which died at birth and another in forty-eight hours. Three young ones lived for three days and the remaining for four days. They were all very feebly developed. Their gonads were as in the above-mentioned young.



A *B*

Fig. 1. Sperms: *A* from a diploid, *B* from a triploid rabbit. Smear preparations stained with iron-haematoxylin (HÄGGQVIST). Magnif. 1000 ×.

Sperm samples from the triploid male contained 763.000 sperms/mm^3. The volume of the ejaculate was normal. The sperm motility was a little reduced.

Sperms were measured in a smear at a magnification of 2000 ×. (Fig. 1.) As control a smear from a normal male rabbit was used. About a hundred sperm heads were measured in each smear, the results obtained being as follows:

		Range of variation	Average	Difference
Length in μ	$2x$:	6,50—8,00	7,15 ± 0,01	1,02 ± 0,06
	$3x$:	7,25—9,50	8,17 ± 0,06	
Breadth in μ	$2x$:	3,50—5,25	4,38 ± 0,01	0,30 ± 0,03
	$3x$:	4,00—5,25	4,68 ± 0,02	

One testis from the triploid male was extirpated at the age of 47 weeks for examination of the chromosomes. At the age of 50 weeks the animal was killed and a more detailed study of the chromosome conditions was carried out. In a subsequent paper in this issue Dr. Y. MELANDER will give a report of these studies.

THE FEMALE.

When it was eight months old the triploid female was brought together with the triploid male and normal males several times. She behaved very aversely. When mounted she cried, ran away and even attacked the males. The little energetic triploid male was never successful in mating her. On the other hand, a robust normal male succeeded in mating her at an age of 46 weeks. Thirty-one days later she brought forth four young, which died immediately at birth. The female, too, died in a little while. Probably this was connected with a highly purulent staphylococcic rhinitis contracted during pregnancy. The illness reduced her appetite and affected her severely. On the whole she seemed to be very liable to infections.

THE ERYTHROCYTES IN THE TRIPLOID ANIMALS.

Another cellular type that is very suitable for examination is the erythrocyte. Blood samples can very easily be taken from the ear veins of the rabbit. FANKHAUSER (1939, 1942, 1945) and GRIFFITHS (1941) have shown that the size of the erythrocytes in *Triturus* varies with the

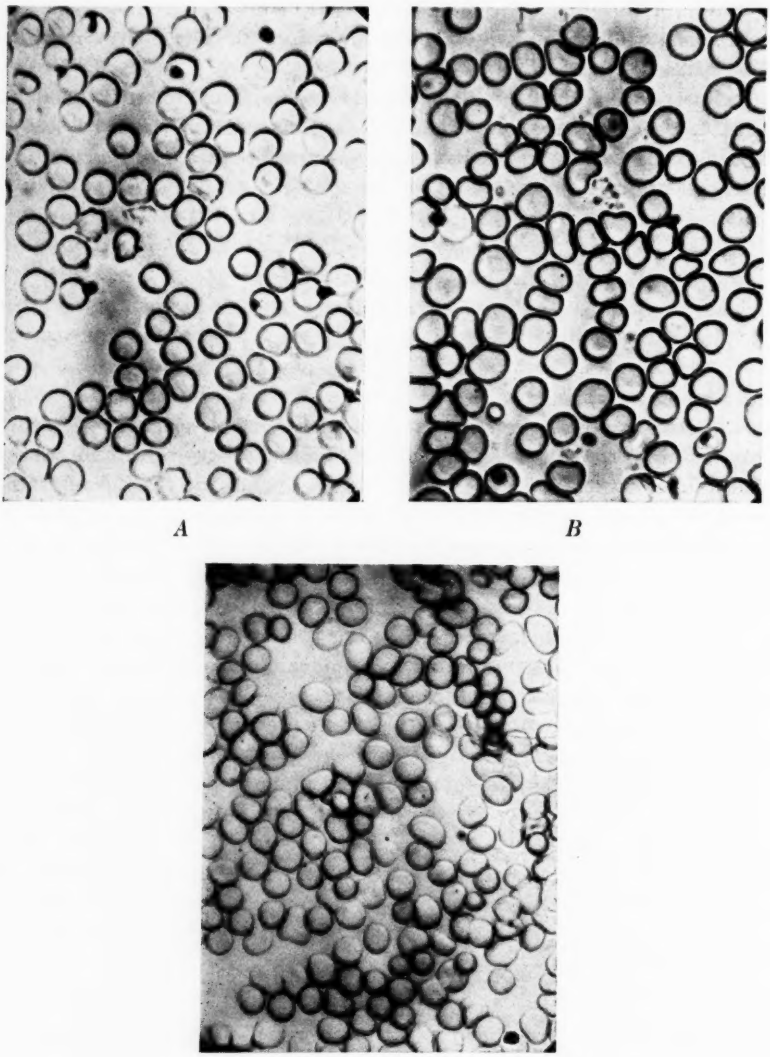


Fig. 2. Erythrocytes. Unstained smear preparations. Magnif. 650 ×. *A* from a diploid rabbit, *B* from a triploid rabbit, 22 weeks old, and *C* from the same rabbit at 51 weeks.

degree of heteroploidy. Contrary to the cells of most organs the erythrocytes undergo no variation in size between day and night.

In new-born rabbits as in new-born humans the erythrocytes are bigger than in adult animals. After birth, the size decreases and from about the sixth week it is constant. The average diameter of erythrocytes in adult rabbits is 6,7 μ. (SCARBOROUGH, 1930—31.) The smears were prepared so thin that the erythrocytes did not cover each other; they were dried in air and measured at a magnification of 1000 × without staining. About a hundred cells were measured in each smear.

To control the method we measured three smears from normal rabbits taken at different times and found the mean $6{,}67 \pm 0{,}03$ μ. (Fig. 2 *A*.)

From the triploid male rabbit four samples were taken between the age of 6—22 weeks. The mean was $7{,}43 \pm 0{,}03$ μ. Range of variation: $7{,}32 \pm 0{,}05$ μ—$7{,}59 \pm 0{,}06$ μ.

From the triploid female rabbit there were taken four samples, too, at corresponding times. Range of variation: $7{,}35 \pm 0{,}06$ μ—$7{,}75 \pm 0{,}06$ μ. The mean was $7{,}53 \pm 0{,}02$ μ (Fig. 2 *B*).

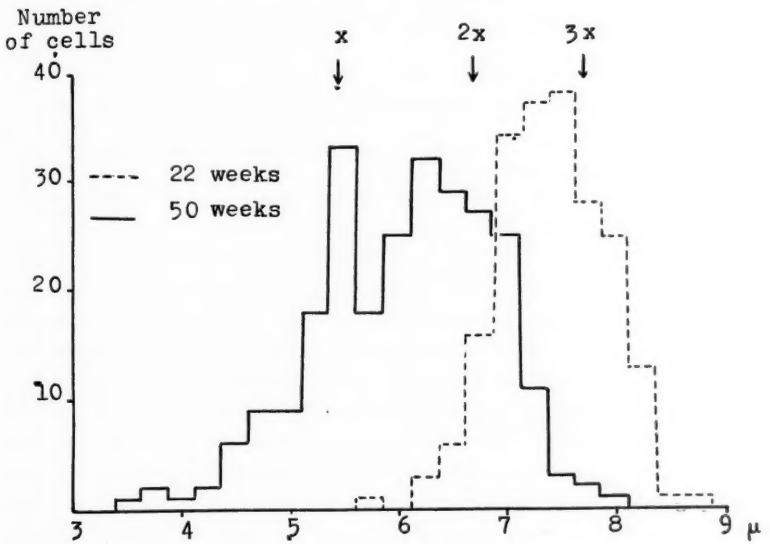


Fig. 3. Distributions of erythrocyte diameters of triploid female rabbit at the age of 22 weeks (dotted line) and 51 weeks (drawn line). Mean diameters of haploid, diploid and triploid cells at arrows.

The volume-ratio of erythrocytes in the male and the female triploid rabbits as compared with those of normal animals is respectively 1,34 : 1 and 1,40 : 1. This value is a little lower than the theoretically calculated 1,50 : 1. The mean of the triploid animals, however, is decreased on account of the fact that in their blood a number of very small erythrocytes occur, one of which can be seen in Fig. 2 *B*. This number was at that time not high. The distribution of the cell diameters can be seen in Fig. 3.

At the age of 51 weeks another blood sample was taken from the triploid female. The blood picture was then found to have changed character entirely (Fig. 2 *C*). 254 cells were measured. Big triploid cells are very few in number. The small cells have increased highly in number and amount to about 7 % of the total number. Most of them consist of cells which have diameters corresponding to those of diploid and haploid cells respectively as well as values between them (Fig. 3). Their mean diameter, therefore, is $6{,}06 \pm 0{,}31\ \mu$, whereas the diameter of haploid cells should be $5{,}38\ \mu$ and that of diploid cells, as mentioned above, $6{,}7\ \mu$. Their volume as compared with that of haploid cells gives a ratio of 1,48 and as compared with that of triploid cells of 0,49.

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CHROMOSOME BEHAVIOUR OF A TRIPLOID ADULT RABBIT

AS PRODUCED BY HÄGGQVIST AND BANE AFTER COLCHICINE TREATMENT

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RECENTLY, HÄGGQVIST and BANE (1950 a, b and c) briefly reported some very interesting experiments with colchicine treatments of rabbit eggs at the time of fertilization. Several adult animals originated after such treatments, some of them showing clear indications of being triploids (bigger cells, *etc.*; see the above-mentioned papers). In the present paper some preliminary data are given concerning the chromosome situation of one of these animals, an adult male killed at the age of 11 months, which I had the opportunity of studying cytologically. This male is also dealt with in the preceding paper; HÄGGQVIST and BANE, 1950 c.

I owe many thanks to Professor Dr. HÄGGQVIST and Dr. BANE for making this investigation possible and also to Dr. A. LEVAN for valuable criticism and suggestions.

The testicles, several ribs with red bone marrow and part of the small intestine were examined.

The ordinary chromosome number of the rabbit was found by PAINTER (1926) to be $2n = 44$. Other authors, including myself, agree with that determination. Thus, there are 2×21 autosomes $+ X + Y$ in the diploid male. (In some rabbits I have seen 1—4 supernumerary minute chromosomes whose real nature is still obscure.)

In the testicles of the present animal there are found several triploid mitotic divisions ($2n = 66$). Hence the meiotic prophase often shows more than 22 units, and at pachytene univalents are observed which, however, often seem to pair with themselves. Further, in some meiotic prophases three nucleolar chromosomes were seen to be attached to the nucleolus. (These chromosomes may be easily distinguished since they are provided with a deeply stained knob at the end opposite the terminal nucleolar organizer.) The first meiotic metaphase of a triploid cell gives a strikingly different impression from that of a diploid cell,

which at this stage shows a very regular formation of bivalents. Contrary to this regular behaviour of the diploids, univalents and trivalents are found at the metaphase of the triploid cells.

Diploid and aneuploid cells, which occur in the testicles of the triploid, too, are commonly present in the ribs and the gut of this animal. Thus, there is a clear difference between the frequency of triploid cells in the testicles and in the somatic tissues studied. This mixture of triploid, diploid and aneuploid cells was really a very unexpected find. The different chromosome numbers found in the various tissues studied are collected in Table 1.

TABLE 1. *The chromosome numbers in various tissues.*

Within the 33—44 classes the uncertainty is ± 1, within the 44—66 classes it is ± 2 owing in some cases to counting difficulties. However, mostly the exact chromosome number could be determined. The pairs of II-M often had somewhat more than 66 chromosomes together, presumably owing to a previous division of univalents.

Chromosome number:	11	17	22	28	33	39	44	55	66	132 ± 5
Bone marrow (ribs). Number of mitotic divisions:			1	4	36	4	31	4		1
Testicle (squash prep.). Number of mitotic divisions: ..	1	1					30	3	7	
Number of II-M pairs:									13	

Preliminary studies of the chromosome situation of the small intestine suggest that it is similar to that of the bone marrow.

In the ribs of a diploid male I found 26 mitotic divisions having 44 ± 1 chromosomes and one with 37 chromosomes.

As seen from the table, a striking feature is that the chromosome numbers of most cells are exact multiples of 11 or numbers close to these multiples. This situation is analogous to what SVÄRDSON (1945) found in embryos of salmonid fishes and VAARAMA (1949) in *Ribes nigrum*.

In order to explain these peculiar chromosome conditions one is entitled to suppose that the embryo of this particular rabbit was actually triploid at the start of its embryonic development, an assumption which is also most likely from other points of view. The egg was treated with colchicine at the moment of fertilization, when the second meiotic division normally is going on. Thus, probably this division was affected by the colchicine with the result that the chromosome number of the egg was doubled. Through fertilization with a spermatozoon having the normal chromosome number a triploid zygote was formed.

Recently, BEATTY and FISCHBERG (1949) as well as FISCHBERG and BEATTY (1950) reported that in several cases the blastulae of mice became triploid or tetraploid if the fertilized eggs were treated at an early stage with abnormal temperatures. However, to my knowledge they have not yet reported anything on polyploid full-grown animals.

Another strong indication of pure triploidy at an earlier developmental stage in the present rabbit case is the fact that the red blood corpuscles studied by HÄGGQVIST and BANE (1950 c) were half a year ago larger than normally and of a homogeneous size. A sister to this male rabbit, which earlier had erythrocytes of a similar appearance, later on had erythrocytes of varying size, and among them several which were even smaller in size than is the case in diploids (see Fig. 3 in the preceding paper by HÄGGQVIST and BANE, 1950 c). This corresponds well to the chromosome conditions actually found in the present male rabbit, although haploid cells seem to be more common in the female.

If the animal was a pure triploid from the beginning all other chromosome numbers, of course, originated later on, during the ontogeny of the animal. As far as I can see, this is the only acceptable explanation of the present chromosome conditions.

However, no simple mitotic mechanism is known to be able to transform triploid cells into diploid ones or, generally speaking, effect a transition from a higher ploidy stage to a lower one. On the other hand, it is well established that the chromosome number of the mammalian cells may be very variable (endopolyploidy being left out of account). This may be due to several different kinds of disturbances of the mitosis. One of the most frequently occurring and the most effective cause of unequal distribution of the chromosomes is multipolar spindles. It is quite feasible that the interior genic balance may cause a disturbance of the mechanism of cell division, as is well-known from new plant polyploids and has actually been observed, for instance, in high-polyploid *Phleum* (LEVAN, 1949). Thus, differences in timing between the divisions of the centrosomes and that of the chromosomes may cause the formation of multipolar spindles. (See SVÄRDSON, 1945.)

In fact, the chance is very minute that two complete sets of chromosomes (2×22) will be gathered to one of the poles during a multipolar mitotic division of a triploid cell (3×22). Nevertheless, the number of cell divisions within a homothermal animal is enormously high and may suffice to account for those events repeated during the lifetime of an animal. The crucial point is whether multipolar spindles

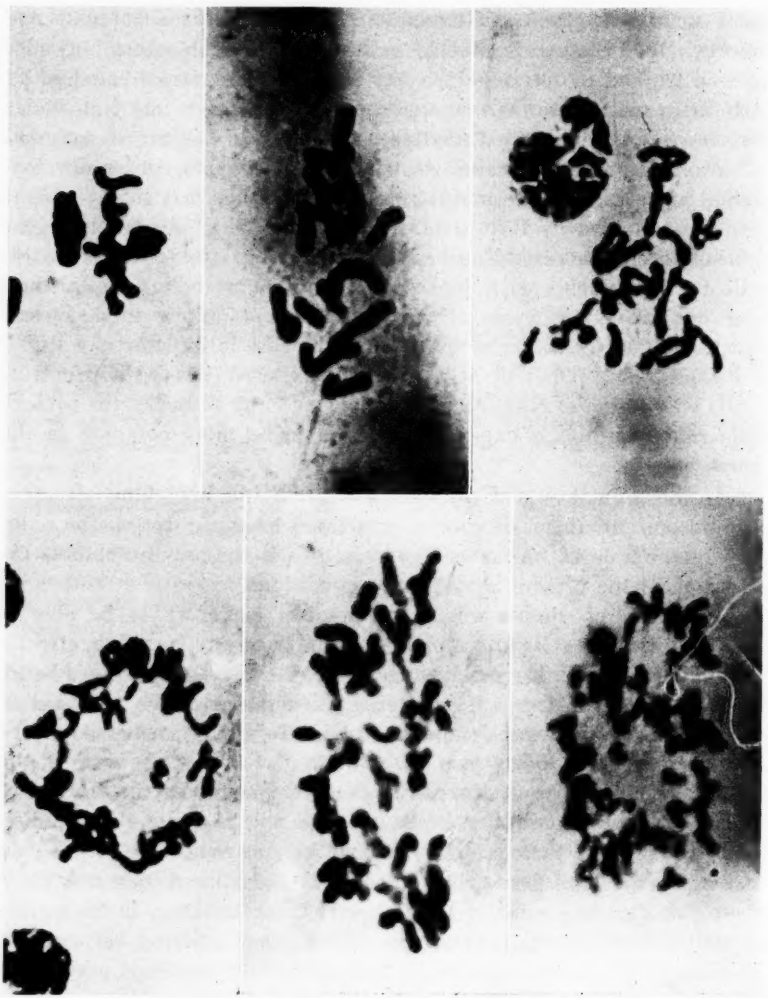

Fig. 1. Cells with 11, 17, 22, 33, 44, and 66 chromosomes from the male rabbit. Microphotos.

are formed frequently enough to allow the origin of the balanced chromosome groups. The study of the blood picture may give information as to the possible occurrence of any developmental period especially rich in such disturbances.

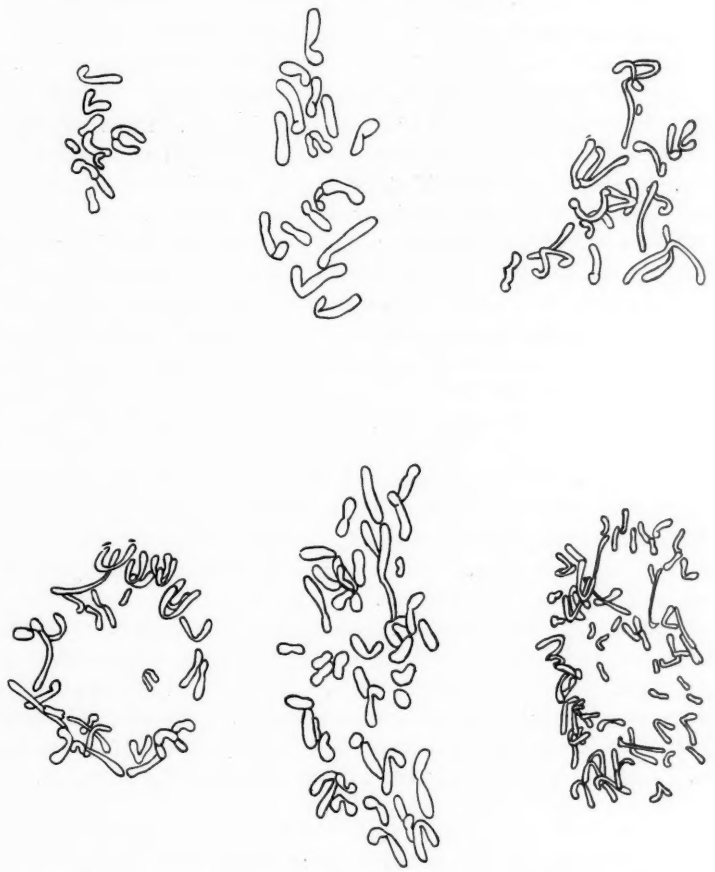

Fig. 2. Cells with 11, 17, 22, 33, 44, and 66 chromosomes from the male rabbit. Camera lucida drawings.

Higher plants are probably fundamentally different from homothermal animals in the respect that the majority of the cells in a plant organ are functioning throughout its lifetime, whereas in many organs of the animals mentioned there is a rapid renewal of cells.

Evidently, if diploid cells originate within a newly produced triploid, these diploid cells are better balanced than the triploid ones and are able to function more satisfactorily. Thus, diploid cells in those

cases probably always have a selective plus value to triploid ones. They will therefore rapidly gain at the expense of the triploid cells. It is well known to plant breeders trying to induce tetraploidy in a diploid meristem that remaining diploid cells may outgrow the doubled cells, which very soon will result in a purely diploid tissue. In this connection I may also refer to the case mentioned by HUSKINS and CHOUINARD (1950). They found tetraploid, triploid and diploid root tips on one single, originally tetraploid specimen of *Rhoeo discolor*.

The aneuploid chromosome numbers which are multiples of 11, *viz.* 33 and 55, are not easily explained. Obviously, however, they are better balanced than other numbers. This undoubtedly points to a change to polyploidy in the previous history of the domestic rabbit, but it should not be denied that there are several objections to such a view.

It is suggestive that the only numbers deviating from the 11-series are the numbers 17, 28 and 39, *i. e.* 5 chromosomes less than 2, 3 and 4 times 11, respectively.

The lacking mitotic balance met with in the present rabbit case constitutes an additional obstacle to the establishment of polyploidy in a population of mammals. Future experiments may settle that interesting question. It may be possible to produce polyploid genotypes with better balanced mitotic behaviour.

The fact that the rabbits of HÄGGQVIST and BANE in all probability start as pure triploids of both sexes has remarkable theoretical and practical consequences, which at the present time can be but fragmentarily surveyed. Much combined experimental and cytological work is obviously necessary in this important field, the result of which may actually bring the mammals within reach of polyploidy breeding.

SUMMARY.

The chromosomes of an adult male rabbit were examined. The animal originated in the experiments of HÄGGQVIST and BANE after colchicine treatment of the egg at the moment of fertilization.

It was found that the testicles contained a mixture of triploid ($2n = 66$), diploid ($2n = 44$) and aneuploid cells and that cells of somatic tissue were mostly diploid or aneuploid. The animal evidently started its development as a pure triploid. During its ontogeny other competitive chromosome numbers arose, possibly as a result of irregular chromosome distribution owing to multipolar spindles or other mitotic disturbances. Those chromosome numbers which are multiples of 11 are most common.

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CASES OF ASYNDESIS IN MATRICARIA INODORA AND HYOSCYAMUS NIGER

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I. INTRODUCTION.

SINCE BEADLE and MCCLINTOCK (1928) and BEADLE (1930) for the first time described a genically controlled case of asyndesis in maize, and EKSTRAND (1932) and SAPEHIN (1933) obtained a similar result with barley and common wheat, the number of cases more or less similar to these have greatly increased. The genetical basis of the phenomenon has been proved many times to be the result of a single recessive gene. An exception of particular interest is provided by the hybrid *Gossypium* described by BEASLEY and BROWN (1942). Here asyndesis is caused by the presence of two complementary genes brought together by hybridization.

These mutated genes whose main effect appears as a failure of chiasma formation are members of a greater gene complex that regulates the normal course of mitosis and meiosis. SVÄRDSON (1945) has given the name »mitotic genes» (*cf.* also VAARAMA, 1949) to the genes of this complex.

In the doubly recessive condition the mutated genes lead to disturbances in the meiotic division mechanism which finally result in a more or less pronounced sterility. Provided that sterility is not complete there may exist descendants of asyndetic forms that originate from gametes changed numerically and/or structurally. This may considerably increase the genotypical variation in the species populations. As the frequency of the genically controlled asyndetic forms seems, in the light of the many new finds, to be fairly common in different groups of Phanerogams — such forms even occur in the animal kingdom (BÖÖK, 1945) — there is reason to consider inherited asyndesis as an evolutionary factor of considerable importance.

Many investigators of asyndetic plants have made attempts to explain the real mechanism of the failure in chiasma formation. All the explanations so far offered must, however, be considered as more or less hypothetical. Neither are the conclusions expressed in the

present paper able to extend our knowledge to any new level. We may in principle agree with the statement of EKLUNDH EHRENBERG (1949, p. 23): »But as long as the problem of chiasma formation remains unsolved, all theories concerning the nature of phenomena which prevent this formation must necessarily be rather hypothetical». The analysis of the asyndesis phenomenon makes possible a better understanding of many of the forces regulating the course of normal meiosis (*cf.* BRIEGER, 1934). The fact that the asyndetic genes are greatly influenced by modifying genes and react readily to extrinsic factors (GOODSPEED and AVERY, 1939; LI, PAO and LI, 1945) makes the analysis difficult, however. In addition, it is characteristic of mitotic genes that a disturbance in the function of one of the factors of the balanced system necessarily causes disturbances in others (*cf.* VAARAMA, 1949). This, of course, makes it difficult to separate observations on the different factors. Every analysed case of asyndesis, however, provides additional material that will assuredly be found valuable whenever the analysis can be made on a more exact basis than is possible at present.

II. MATERIAL AND METHODS.

The asyndetic form of *Matricaria inodora* ($2n = 36$; *cf.* LÖVE and LÖVE, 1948) was found growing in the fields of the State Horticultural Institute in the late autumn of the year 1948. One plant drew attention because of its slenderness and the unusually small size of its flowers. The investigation of the fixed material was carried out during the following winter, when the asyndetic condition of the plant was also detected. The collecting of seed failed in the autumn. No more individuals of this annual plant were found the following summer. The present investigation is therefore based solely on this single plant specimen.

A trial plot of *Hyoscyamus niger* was grown in the Experimental Garden of the State Horticultural Institute in the summer of 1948. The seed was from the Botanical Gardens of Helsinki, Finland. Among the plants growing in the trial plot there were a considerable number, about 5—8 % of the total, of completely sterile individuals. A number of sterile plants were again obtained the next summer when the trial was renewed using seed taken from the plants grown the previous year. The sterile plants all proved to be asyndetic.

In both the species mentioned only microsporogenesis has been investigated and is described in this paper.

The fixation of the buds of both the species was carried out by

dipping them first for 10—20 seconds in acetic acid-alcohol (1 : 3) and then transferring to CRAF-solution (according to WEBBER's schedule). Particularly with *Hyoscyamus* the result obtained was excellent.

The fixed material was imbedded in paraffin. The slides were stained using the usual iodine gentian-violet method. In studying meiosis in the normal *Hyoscyamus* plants, however, the aceto-orcein squash method was used.

III. RESULTS.

1. MATRICARIA INODORA.

Prophase. — In *Matricaria inodora* asyndesis appears at a rather late stage of meiosis. In most cases 22 units are observed at diakinesis (Fig. 1). One can thus expect that only four possible bivalents remain as univalents. The presence of so high a number of bivalents as 14 at first metaphase is, however, unknown. We have, therefore, reason to assume that the separation of univalents continues after diakinesis, apparently during prometaphase. There exists a certain resemblance to the asyndetic *Secale* (PRAKKEN, 1943) in which the formation of univalents takes place during approximately the same stage of meiosis.

First metaphase. — There exists usually only one spindle at first metaphase. This includes all bivalents as well as univalents. Only seldom may a few univalents be observed outside the spindle. All the bivalents and a varying number of univalents proceed to the equator, the remaining univalents being scattered inside the spindle (Figs. 2 and 3). The number of bivalents has decreased considerably since diakinesis. The frequency of bivalents as counted from 20 PMCs is as follows:

Number of bivalents						Total	Mean
3	4	5	6	7	8		
3	2	4	6	3	2	20	5,5

Accordingly, on an average one-third of the chromosomes have formed true bivalents. The majority of bivalents are rod-shaped. In 25 per cent of the PMCs, however, a single ring bivalent has been found. It seems probable that the chiasma frequency has somewhat decreased, because in the normal plants in about 80 per cent of the PMCs 1—3 ring bivalents are found.

The bivalents of the asyndetic form are abnormally long and stretched (Fig. 3). The same phenomenon has been observed also in many other asyndetic plants (*cf.* BEADLE, 1930; KOLLER, 1938; JOHNSSON,

Figs. 1—9. *Matricaria inodora.* — Fig. 1. Diakinesis with 8 univalents and 14 bivalents. — Fig. 2. I-M side view with 5 bivalents. — Fig. 3. Early I-A with 6 elongated bivalents. — Fig. 4. I-A side view. A number of univalents divide late. — Fig. 5. I-A. Misdivision and fragmentation of the divided units can be observed. — Fig. 6. I-T. Eliminated univalents and fragments form micronuclei. — Fig. 7. II-M. Two metaphase plates. Univalents divided at I-A lie scattered inside the spindle. — Fig. 8. II-A. A bridge in the right hand figure. Two fragments can be observed to the left. — Fig. 9. Late II-A with a bridge in the other division figure. — × 2300.

1944; LI, PAO and LI, 1945; EKLUNDH EHRENBERG, 1949). However, no corresponding spindle abnormalities such as bending and multipolarity of the spindle, which seem to be fairly common in asyndetic forms, *e. g.*, *Zea* (BEADLE, *l. c.*), *Datura* (BERGNER, CARTLEDGE and BLAKESLEE, 1934), *Pisum* (KOLLER, *l. c.*), and *Rumex* (LÖVE, 1944), have been found.

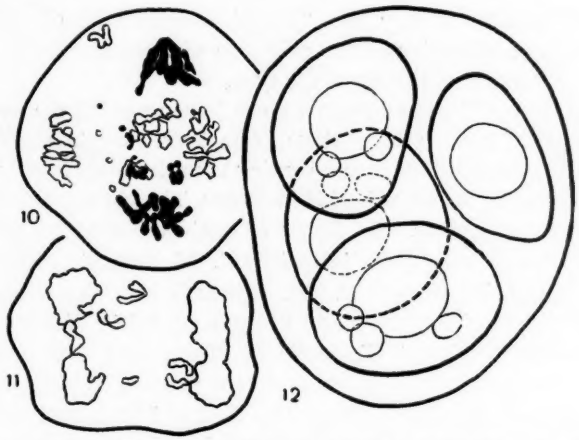
First anaphase and interkinesis. — At first anaphase the halves of the bivalents move normally towards the poles. There may, however,

occur irregularities in the separation of the halves as indicated by the stretching of the bivalents. This often leads to a fragmentation of the chromosomes (Fig. 4). Owing to the fact that fragments have been observed even before the beginning of anaphase, we can draw the conclusion that the chromosomes can be fragmented also during the separation of units at prometaphase. The chromosomes can apparently break also at any point beside the chiasma (Fig. 5). This makes possible structural changes in the chromosomes, deficiency in one half of the bivalent and inverted duplication in the other. True bridges have not been observed at the first ana-telophase.

It is a regular occurrence that after the separation of the bivalents a number of univalents divide at the equator (Figs. 4 and 5). There is a great variation in this respect between different asyndetic forms described in the literature. The division of univalents at first anaphase has been met with more or less frequently in asyndetic forms, *e. g.*, *Hordeum* (EKSTRAND, 1932), *Rumex* (YAMAMOTO, 1934), *Crepis capillaris* (RICHARDSON, 1935), *Pisum* (KOLLER, 1938), *Oenothera* (CATCHESIDE, 1939), *Secale* (PRAKKEN, 1943), *Musa* (DODDS, 1943), and *Triton* (BÖÖK, 1945). The division of univalents does not seem to be correlated with the strength of the asyndesis. It is probable that the univalents also sometimes suffer misdivisions during this stage.

The formation of restitution nuclei has not been observed at this stage of meiosis. Two nuclei are regularly formed at the interphase. A few univalents and fragments remain lagging at first anaphase, giving rise to the formation of degenerating micronuclei (Fig. 6).

Second division. — At second metaphase two regular plates have been formed by the halves of the bivalents and by the univalents that remained undivided during first anaphase (Fig. 7). The divided univalents, on the other hand, will be found scattered in the spindles. Many of them remain as laggards at second anaphase (Figs. 10 and 11). Particularly characteristic of the second anaphase are the bridges, which are mostly true bridges, although it is not always possible to discern the acentric fragments (Figs. 8 and 9). Never has there been observed more than one bridge in either of the second anaphase spindles. The formation of bridges is a fairly common phenomenon in the asyndetic plants so far investigated (*cf.*, *e. g.*, KOLLER, 1938; LAMM, 1945; ANDERSSON, 1947). The bridge formations described differ, however, from those of the present case in that bridges have always been observed in the anaphases of the both divisions. The bridges of the first division are here lacking or at least they must be quite rare in *Matricaria*.



Figs. 10—12. *Matricaria inodora.* — Fig. 10. II-T with many fragments and eliminations. — Fig. 11. II-T. Two of the chromosome groups forming a restitution nucleus. — Fig. 12. Tetrad stage. All the micronuclei included within the large pollen grains. — × 2300.

Fragmentation occurs, not only in connection with bridges, but also when the other chromosomes suffer a misdivision. A total of six fragments can be clearly seen in Fig. 10.

As as rule, four macronuclei are formed at the second anaphase and also additional micronuclei originating from lagging chromosomes and fragments. Restitution nucleus formation between two of the telophase chromosome groups has been observed in only few cases (Fig. 11). The tetrad, however, consists of four cells, the size of which varies owing to the irregular distribution of the chromosomes during the first and second divisions. The micronuclei do not form separate pollen grains because they always remain inside the larger ones (Fig. 12). This is quite a characteristic feature in *Matricaria* and has been observed also in the microsporogenesis of the triploid hybrid *M. maritima* × *inodora* (VAARAMA, unpubl.).

2. HYOSCYAMUS NIGER.

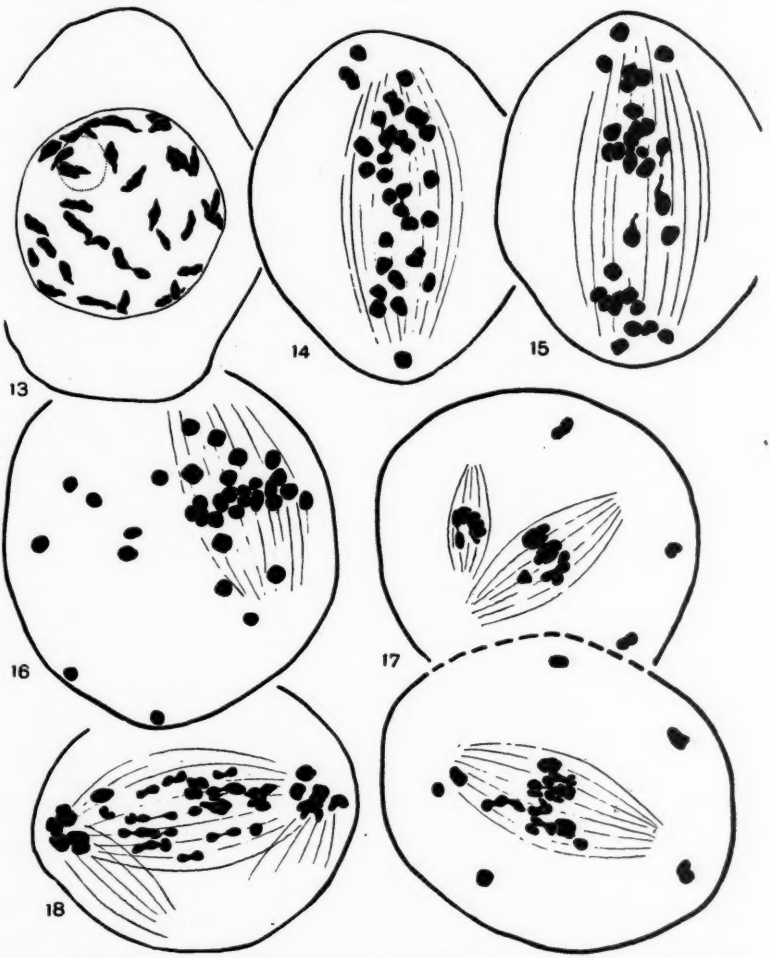
Mitotic divisions. — Mitoses of the asyndetic *Hyoscyamus niger* have been compared with the mitoses of the normal type (seed from the Botanical Garden of Basle, Switzerland). No mitotic disturbances have been observed in the asyndetic plants. Nevertheless, the maximum number of nucleoli in the asyndetic plants proved to be three, whereas

the normal number is only two. This phenomenon might perhaps be explained as an indication of the existence of some structural changes in the chromosomes of the asyndetic type.

Prophase. — The observations made have revealed the occurrence of univalents at a very early stage of meiosis. Certain stages of the prophase, apparently those corresponding to pachytene, closely resemble those described by RAMAER (1935) in *Hevea*. The chromosomes mainly appear as single threads. The degree of conjugation, as far as it exists at all, is at any rate quite insignificant. 34 separate units can be distinguished at diakinesis (Fig. 13). Asyndesis is quite complete, as in *Datura* (BERGNER, CARTLEDGE and BLAKESLEE, 1934), *Hevea* (RAMAER, 1935), *Godetia Whitneyi* (HÅKANSSON, 1943), and *Solanum Rybini* (LAMM, 1945). Cases of almost complete asyndesis, bivalent formation being quite rare, have been reported by PAL and RAMANUJAM (1940) in *Capsicum annuum*, by JOHNSSON (1944) in *Alopecurus myosuroides*, and by BÖÖK (1945) in *Triton*.

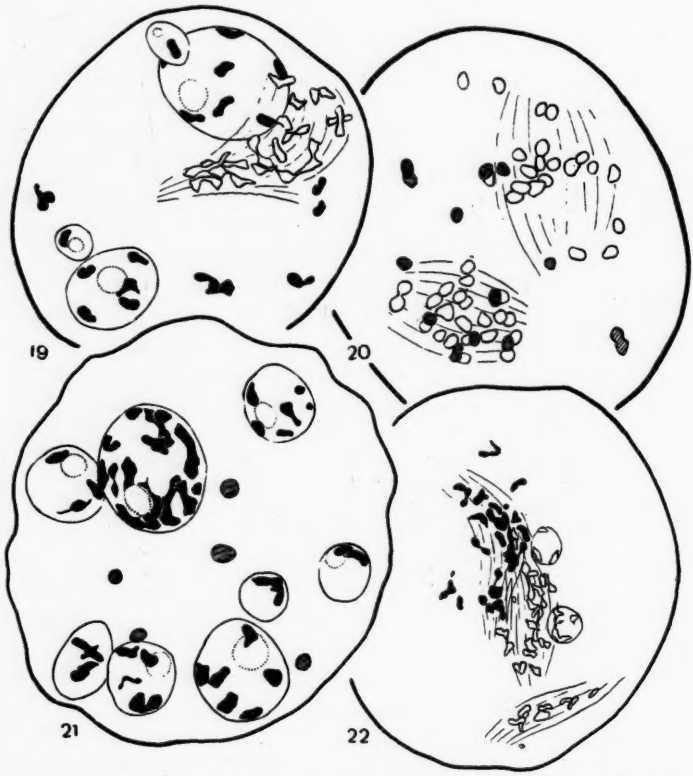
First metaphase. — In the different anther lobes the formation of the metaphase figure takes place in various ways. In some lobes the univalents are scattered at random in the spindle so that »they run like a band through the whole cell», as RAMAER (1935, p. 222) described this phenomenon in *Hevea* (Fig. 14). The spindle is then abnormally elongated and narrow, in a few cases also bent. Sometimes an indistinct grouping can be observed around the equatorial plate, the ordinary metaphase plate being, however, absent. A varying but small number of univalents may remain outside the spindle, forming supernumerary spindles. These are, however, rare in this type of meiosis. As may be seen in Fig. 14, there are in the metaphase figure associations of univalents resembling bivalents. These apparently are not true bivalents, but are caused by the stickiness of the chromosomes. This view is supported by the fact that chains of numerous univalents may also appear. Similar false bivalents have also been observed in several other completely asyndetic plants (RAMAER, 1935; LAMM, 1945).

In addition, in this type of meiosis univalents with appendages resembling satellites are observed (Fig. 15). These are always directed towards the equatorial plane. LAMM (*l. c.*), who described similar univalents in *Solanum Rybini*, explained them as evidence of the T-phenomenon, *i. e.*, the existence of a secondary centromere (PRAKKEN and MÜNTZING, 1942). It seems, however, more probable that this is not a question of the T-phenomenon but of false bivalents recently separated



Figs. 13—18. *Hyoscyamus niger*. — Fig. 13. Diakinesis with 34 univalents. — Fig. 14. I-M. The type with scattered chromosomes showing several pseudo-bivalents. — Fig. 15. I-M. Three univalents provided with a satellite-like appendage. — Fig. 16. I-M. The type with metaphase plate formation. Observe the displaced plate. — Fig. 17. I-M with three separate spindles. Anaphase is just beginning in the bottom-most spindle. — Fig. 18. I-A. Dividing and non-dividing univalents between the poles. — × 2300.

from one another. It must, however, be mentioned that the T-phenomenon is very clear in the asyndetic *Secale* (PRAKKEN, 1943).



Figs. 19—22. *Hyoscyamus niger.* — Fig. 19. Interkinesis showing the lack of synchronization between divisions in the different spindles. — Fig. 20. II-M Two metaphase plates. The shaded chromosomes lie within the cytoplasm. — Fig. 21. Interkinesis with 8 nuclei. The shaded chromosomes are single univalents eliminated at I-A. — Fig. 22. II-A. Observe the defectiveness of the divisions. Fragmentation occurs abundantly. Two telophase nuclei are already formed. — × 2300.

The other type of first metaphase is characterized by the formation of a distinct metaphase plate (Fig. 16). A varying number of univalents (in 25 analysed PMCs 9—33, average 20,6) have formed a more or less regular plate. The remaining univalents lie scattered inside the spindle or outside it in the cytoplasm. The formation of several spindles is a rather common event (in about 20 per cent of the PMCs). Their axes are situated at an angle to each other (Fig. 17). The spindles are relatively short but are mostly located more or less asymmetrically inside the cell (Fig. 16).

First anaphase and interkinesis. — Some of the univalents divide during first anaphase (Fig. 18). This occurs both in the type where univalents are scattered (*cf.* CATCHESIDE, 1939) and in the type where the metaphase plate is regularly formed. It is obvious that misdivisions of univalents sometimes occur leading to fragmentation and structural changes. The movement of the half chromatids towards the poles is abnormally slow and the formation of interkinesis nuclei often begins before anaphase has been completed. When the PMC contains more than one spindle the lack of synchronization between the different chromosome groups becomes particularly evident (Fig. 19). Anaphase still continues in one spindle while the other have reached interphase. The formation of restitution nuclei has not been observed although it might be expected. In the scattered metaphase type a row of separate nuclei arises. In the univalents scattered in the cytoplasm a clear tendency to divide can be observed during anaphase (Figs. 17 and 19). They frequently give rise to the formation of micronuclei. A few univalents, however, degenerate.

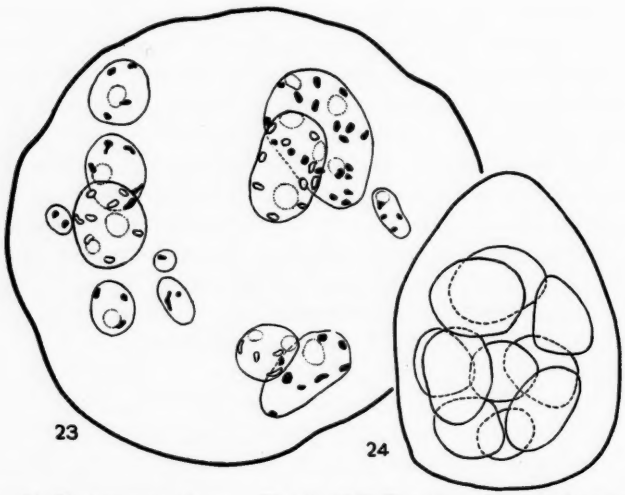
At interkinesis the PMC usually contains numerous nuclei (Fig. 20). The average for each cell as counted from 25 PMCs was 7,4 (3—11). Not all the nuclei contain a nucleolus. The most common number of nucleoli in a nucleus is one, the numbers 2 and 3 being more rare. The number of nucleoli in a PMC was on an average 5,2 (2—8).

Second division. — Metaphase plates are always formed at second metaphase. The plates are never, however, quite regular. Most chromosomes form a more or less uneven group. The remaining chromosomes lie scattered either inside the spindles or in the surrounding cytoplasm (Fig. 21). The average count for 25 PMCs shows that there are 33,6 chromosomes within the plates and 15,6 chromosomes outside. Two is the most common number of second division spindles, the variation, however, being as stated from one to four. The frequency of the different numbers of spindles as counted from 50 PMCs is as follows:

Number of spindles				Total
1	2	3	4	
8	35	6	1	50

Because the number of interkinesis nuclei is great it seems that there occurs a fusion of spindles, the smaller spindles being included in the larger ones (*cf.* LEVAN, 1940).

A part of the chromosomes situated in the plates divide. The ana-



Figs. 23—24. *Hyoscyamus niger.* — Fig. 23. II-T. The 12 nuclei contain a total of 71 chromocentres. × 2300. — Fig. 24. »Tetrad» stage with 10 pollen grains. — × 1200.

phase movement of the chromatids seems to be defective; the well-defined approach to the poles entirely fails (Fig. 22). The situation thus somewhat resembles first anaphase. Neither has the formation of a restitution nucleus been observed at second anaphase. Instead, a group of separate nuclei appears. In addition the scattered chromosomes form separate micronuclei. The counts made on 25 PMCs have shown that there are an average of 13,8 nuclei per PMC. At the same time the number of nucleoli was found to be 11,4 (Fig. 23). Compared with the number at interkinesis the number of nucleoli has approximately doubled.

It is impossible to estimate by ocular investigation whether all the univalents that divided at first anaphase remain undivided at the second division. The possibility of double univalent division, which has been observed in the hybrid *Ribes Gordonianum* by MEURMAN (1928), had to be taken into consideration. It was, however, possible to establish statistically that the double division does not occur in *Hyoscyamus.*

Statistics have been calculated from a single anther lobe where it was possible to find a sufficient number of different division stages from first metaphase to second telophase. The numbers obtained are thus comparable with each other. The counts have been made in each case on 25 PMCs.

Division stage	Mean Units in the plate	Units outside the plate	Total
First metaphase	20,6	13,7	34,3
Interkinesis	—	—	45,6
Second metaphase .. .	33,1	15,6	48,7
Late second telophase ..	—	—	71,3

If we take as a starting point the fact that all the univalents, on an average 20,6, lying in the metaphase plate divide at first metaphase, we should expect to find 54,9 units in the interkinesis nuclei. Because on an average only 45,6 units were established, this means that 9 units on an average remained undivided in each metaphase plate. On the similar assumption that all the units in the second metaphase plates divide, the total number of units in all second telophase nuclei should be 81,7. The counts, however, show that there are on an average only 71,3 units, the average number of undivided units in the plates being thus 10,4. We can summarize the results as follows:

Division stage	In the plates dividing units	non-dividing units	Total of non-dividing units
First metaphase	11,6	9,0	22,7 (9,0 + 13,7)
Second »	22,6	10,4	26,0 (10,4 + 15,6)

The summary shows that the total number of non-dividing units at first metaphase and the number of dividing units in the plates at second metaphase correspond well to each other. The number $2 \times 11{,}6 = 23{,}2$, *i. e.*, the number of units formed in the division of the univalents at first anaphase and the number of non-dividing units at second anaphase are in good accordance with one another. The conclusion may thus be drawn that the univalents which divided in the first division do not divide in the second division.

All the nuclei appearing at second telophase cannot form separate pollen grains. In spite of this the number of grains at the tetrad stage is very great, varying from 4 to 12 (Fig. 24). The most common number of pollen grains formed is 7—9. The sterility of the pollen is apparently complete.

Macrosporogenesis has not been investigated in this case but there is reason to suppose that it is disturbed like the microsporogenesis. Seed formation was not established in the asyndetic plants of *Hyoscyamus niger*.

IV. DISCUSSION.

1. THE NATURE OF ASYNDESIS.

With regard to the degree of asyndesis the former of the plants described, *Matricaria inodora*, represents a typical case of medium strong asyndesis, the latter again, *Hyoscyamus niger*, a case of complete asyndesis (PRAKKEN, 1943, p. 487). As to the position of the plants described in the classification of PRAKKEN (*l. c.*) according to the origin of asyndesis, we have reason to believe that both the cases belong to PRAKKEN's first category, *i. e.*, asyndesis is caused by a certain gene or genes.

With regard to *Matricaria* there does not appear to exist any direct evidence supporting this assumption. It seems, however, less probable that asyndesis could have been caused by external factors alone. The plants growing in the close neighbourhood of the asyndetic individual were normal. The chromosome number is normal, the possibility of asyndesis caused by nullisomy (*cf.* HÅKANSSON in *Godetia*, 1943) being thus excluded. Neither is there any question of a partially sterile species hybrid. This is seen both from the morphology of the individual and from the fact that all the *Matricaria* species that could be considered to be the other hybrid parent were diploid and would thus have given rise to triploid hybrids. Genically controlled asyndesis seems to be the only plausible mode of asyndesis which can be considered likely in the present case.

True bridges were commonly met with at second anaphase, indicating the presence of structural hybridity. It must, however, be quite a rare phenomenon in the normal *Matricaria inodora*. In spite of numerous observations, the author has never found cases of it in natural populations of *M. inodora*. It may, therefore, be supposed that the structural hybridity is a result of asyndesis that has occurred already in some of the earlier generations. It may be assumed that one or both of the gametes that have taken part in the creation of the individual in question have undergone an asyndetic meiosis. As has been mentioned earlier, there occur difficulties in *Matricaria* in the separation of the bivalent halves at first anaphase. This leads to a stretching and breakage of the chromatids at wrong points. Thus there may arise a chromosome suffering from a deficiency, and consequently also a chromosome with an inverted duplication at its distal end. It is quite possible that the latter types of structurally changed chromosomes can be transferred, after the healing of the breakage, to the next generation by functioning

gametes. FRANKEL (1949) has found such structural changes in common wheat and has studied the influence on meiosis of inverted segments of various size combined in different ways. When a comparison of the asyndetic *Matricaria* is made with the analyses of FRANKEL (*l. c.*), it seems probable that the inverted duplication segment in *Matricaria* has been short and that the plant has been heterozygous with respect to this segment. The chiasmata formed have apparently been internal between the inverted segment and its normal homologue. This has led to the formation of bridges in the second division.

To what extent the bridges observed in the other asyndetic plants (KOLLER, 1938; PRAKKEN, 1943; ANDERSSON, 1947; EKLUNDH EHRENBERG, 1949) are comparable with the *Matricaria* case is uncertain. The asyndetic *Pisum* individuals studied by KOLLER (*l. c.*) are descendants from seeds of asyndetic forms. The bridges occurring in the first as well as in the second division may, thus, be derived from inversions that have occurred during asyndetic meiosis in the parental generation. It may be stressed once more that the asyndetic *Matricaria* represents the first case among asyndetic plants where bridges have been found only in the second division.

The genically controlled nature of the asyndesis in *Hyoscyamus niger* is more obvious. Sterile asyndetic forms always occur side by side with normal plants. Owing to the occurrence of complete sterility we can take it for granted that the asyndetic individuals must always be repeatedly created by conjugation of gametes heterozygous for the asyndesis gene or genes.

2. THE CAUSES OF ASYNDESIS.

Failure of chiasma formation may be considered to be the ultimate cause of asyndesis. Experiments performed with a view to discovering the factors leading to such a failure have shown that where the homology of the chromosomes offers a possibility of regular conjugation, the cause of a decrease in the number of chiasmata or their total absence may be found in the insufficient precocity of the prophase in meiosis (DARLINGTON, 1937; FRANKEL, 1940; BARBER, 1941). This may be expressed in such a way that the conjugating chromosome threads split before the beginning or completion of the zygotene pairing. DARLINGTON (1937) has termed this phenomenon the time limit of pairing, this term being, however, restricted to cases of interrupted pairing. The splitting of the chromosome threads before the beginning of the pairing is, nevertheless, to be considered only as one aspect of one and the same phenomenon.

BEADLE (1933), in his discussion of the possible causes of asyndesis in *Zea*, has suggested that the effect of asyndetic genes expresses itself as just such an insufficient precocity of the prophase. The conclusions drawn later by KOLLER (1938) and PRAKKEN (1943) have followed along the same lines. The premature splitting of the chromosome threads can without hesitation be accepted as the cause of asyndesis in those cases where clear-cut pachytene pairing cannot be observed (YAMAMOTO in *Rumex*, 1934; HUSKINS and HEARNE in asyndetic fatuoid oats, 1933; RAMAER in *Hevea*, 1935; and the present case, *Hyoscyamus*). Many other cases of asyndesis have, nevertheless, shown that the pachytene pairing may be, at least under ocular examination, complete and that the separation of the univalents appears later on. The separation of univalents may occur even as late as the prometaphase stage (*Matricaria inodora*, the present case). There have been difficulties in the adaptation of cases such as these to the premature splitting hypothesis. Several students of asyndesis (JOHNSSON, 1944; EKLUNDH EHRENBERG, 1949) have, therefore, taken a sceptical attitude to the application of this idea.

There is, however, in the author's opinion, a case in the literature in which the time limit of pairing has been fairly indisputably indicated as being the cause of asyndesis. Thus, DARLINGTON and JANAKI AMMAL (1945) have stated that in a spontaneously occurring triploid asyndetic type of *Nicandra physaloides* the isochromosomes pair normally while the autosomes are strongly asyndetic. There is, of course, a much better chance for the arms of the isochromosomes to pair than for the autosomes that lie more widely separated from one another.

There seems to be little reason to suppose that more than one basic factor is the cause of the asyndetic phenomenon. If the observations strongly support the hypothesis that asyndesis has been caused by the insufficient prophase precocity in one case, the same hypothesis ought also to be valid in every other case. In general, an intergraded series of events has been formed by the cases of experimental and spontaneous asyndesis, the essential differences being in the strength of the phenomenon. The other differences might be interpreted by the many possible modifications of the same factor, *viz.* the relatively late incidence of prophase in meiosis.

The division of the centromeres and the conjugating units are two separate events, as has been emphasized, for instance, by DARLINGTON (1937). These do not need to be correlated in time. If we accept the chiasma formation theory of DARLINGTON (*l. c.*) it may be supposed

that the normal splitting of conjugation units appears at a late stage of pachytene. Equilibrium has then been reached between the hypothetical torsion in and between the chromatids, and splitting automatically causes the formation of chiasmata. The division of the centromeres, on the contrary, still occurs at second anaphase. When insufficient prophase precocity exists discrepancies in the timing of both divisions can occur separately. There are thus many possibilities of combination between the two variously timed disturbances.

Especially disturbances in the timing of the centromere division in experimentally induced asyndetic *Trillium kamtchaticum* have been studied by MATSUURA (1937). His material has been particularly suitable for this purpose owing to the lack of chiasma formation and to the large size of the chromosomes. On the basis of centromere divisions occurring at different stages of prophase, MATSUURA (*l. c.*) has been able to distinguish three different types of asyndetic meiosis: precocious, desynaptic and asyndetic. In the first case, the division of the centromere in the homologous chromosomes appears at the diplotene stage, in the second case at diakinesis, and in the last case before the zygotene stage.

It is characteristic of the asyndesis phenomenon that the prophase precocity is differential with regard to different chromosomes. In case of partial asyndesis precocity is normal in a part of the chromosomes, and a number of bivalents are formed. In the remaining part of the chromosomes, on the contrary, the prophase precocity is insufficient and chiasma formation fails. The time of appearance of the univalents varies greatly, however, in different cases, *i. e.* from pachytene (BEADLE, 1930, *Zea*) to prometaphase (*Matricaria*, present case). The time of separation of the univalents in meiosis is, nevertheless, characteristic of each asyndetic organism.

The splitting of conjugating units has occurred before the beginning of pairing in cases where no normal pachytene thread has been observed. Such cases where the univalents are capable of orientating and dividing in the first division are apparently comparable with the »asyndetic» meiosis type of MATSUURA (1937). There also the division of the centromere has been precocious. In a part of the chromosomes, however, the division of the centromere occurs normally at the anaphase of the second division.

It has been observed that the second metaphase plates in *Hyoscyamus* contain more units than there are undivided univalents in first anaphase (*cf.* above). This can be explained only by assuming the

development at interkinesis of polarization in the centromeres of some univalents that have divided at first anaphase. They are thus capable of auto-orientating themselves in the second division. The chromatids have not, however, divided, the double division of univalents not being realized. Both the necessary conditions for this phenomenon exist, however, in certain cases, as described by FEDERLEY (1913) in *Pygaera* hybrids, by MEURMAN (1928) in *Ribes Gordonianum*, and by SCHRADER (1945) in the hemipterous insect *Loxa*.

The application of the insufficient prophase precocity hypothesis is unquestionably difficult in the numerous cases where it is possible to observe a distinct prophase pairing of chromosomes. This difficulty mainly originates from our too incomplete and inexact knowledge of the mechanism of chiasma formation. This is, however, not a sufficient reason for abandoning the whole hypothesis. If we continuously maintain the theory of DARLINGTON (1937) as the basis for the formation of chiasmata, there is a hypothetical possibility that the splitting of the conjugating units occurs before the fulfilment of the mutual equilibrium between the torsion and relational coiling in and between the chromatids. It is possible that normal chiasma formation will be prevented in such a way. The univalents remain together, however, whether owing to relational coiling (*cf.* KOLLER, 1938; LEVAN, 1940) or to a certain residual attraction of a rather obscure nature. If some of the univalents in such a type of asyndesis divide in the first division we can furthermore assume that the precocious division of the homologous centromeres has prevented the establishment of normal conditions for chiasma formation.

The normal co-orientation of bivalents will be prevented in those cases where pairing and chiasma formation have taken place undisturbed, the centromere having, however, divided precociously. Instead of being co-orientated, the bivalents are auto-orientated, which leads to the morphological post-reduction of the bivalents, as was described in *Odonata* by OKSALA (1943, 1944). The »precocious» type of experimental asyndesis described by MATSUURA (1937) differs from the above-mentioned case only in the fact that chiasmata are lacking.

In connection with the examination of asyndetic meiosis it is necessary also to examine the features of spindle formation. Many students of asyndesis have often reported discrepancies in this respect (*cf.* BEADLE, 1930; BERGNER, CARTLEDGE and BLAKESLEE, 1934; RAMAER, 1935; KOLLER, 1938). With regard to the strength of the

disturbances the asyndetic *Hyoscyamus* seems to exceed all the cases known hitherto.

The spindle disturbances appear above all as a weakening of the polarity factor and an abnormal stretching of the spindle. The upset of the normal concentration of the polarity factor in asyndetic organisms becomes apparent either in the multipolarity of the spindle (LÖVE, 1945; EKLUNDH EHRENBERG, 1949) or in the formation of a split spindle, *i. e.*, in the appearance of several separate spindles (BEADLE, 1930; RAMAER, 1935; KOLLER, 1938). The latter feature is unusually striking in *Hyoscyamus*, and the separate spindles are commonly polarized quite randomly.

In the animal kingdom the stretching of the spindle at first anaphase is a normal event which contributes to the anaphase separation of the chromosomes (*cf.* RIS, 1949). In the plant kingdom this phenomenon is usually less pronounced or absent, and to the extent that it occurs in asyndetic forms it is unquestionably abnormal. The two types of asyndetic first meiotic metaphases in *Hyoscyamus* described earlier, in which the univalents are either partly congregated in the equatorial plate or lie scattered inside the spindle, are apparently to be traced to the various degrees of stretching of the spindle. In the scattered metaphase type the spindle has been stretched during the prometaphase and has prevented the congression of univalents, which without the separating effect of spindle elongation would be capable of orientating themselves.

I wish to acknowledge my gratitude to the Director of the State Horticultural Institute, Professor O. MEURMAN, for the opportunity to carry out this study at the Cytological Laboratory of the Institute.

SUMMARY.

(1) Asyndetic meioses of the PMCs in *Matricaria inodora* and *Hyoscyamus niger* were described. The former represents medium strong, the latter complete asyndesis.

(2) In *M. inodora* ($2n = 36$) the univalents mainly appear as late as prometaphase. The mean number of bivalents formed is 5,5. Some of the univalents divide at first anaphase. The bivalents are abnormally stretched and there probably occur breakages in the chromatids during

the anaphase separation of bivalents. Fragments are fairly common in the first division. Spindle formation is not disturbed.

(3) In the second division two metaphase plates are formed, the univalents that divide at first anaphase behaving as laggards in the second division. The formation of true bridges is characteristic of the second division. Tetrads are regularly formed, micrograins being absent. The pollen grains are of different sizes owing to the uneven distribution of the chromosomes.

(4) In *Hyoscyamus niger* ($2n = 34$) no normal pachytene threads have been observed. 34 univalents are observed at diakinesis. In one type of meiotic metaphase all the univalents are scattered in the spindle. In the other type the number of spindles varies from one to four. Metaphase plates have been formed, a high proportion of the univalents being, however, scattered within the spindles. Some of the chromosomes divide in the first division. The anaphase movement is defective; restitution nuclei are not, however, formed. Fragmentation occurs frequently and spindle disturbances are a remarkably common feature.

(5) The interphase contains 3—11 nuclei. In the second division from one to four more or less irregular metaphase plates are formed. All the univalents, except those that divided at first anaphase, divide in the second division. Anaphase is defective. On an average 13,8 telophase nuclei are formed. The formation of micronuclei is abundant. The number of units at the »tetrad» stage varies from 4 to 12. The sterility of the asyndetic plant is complete.

(6) Evidence supporting the genically controlled nature of the asyndesis has been presented.

(7) The individual of *Matricaria inodora* investigated is probably descended from parents one of which has already been asyndetic. The structural change, probably an inverted duplication joined distally to one chromosome and expressing itself as second division bridges, probably arises from an asyndetic meiosis in the previous generation.

(8) An attempt has been made to apply uniformly the hypothesis of insufficient prophase precocity to all cases of genically controlled asyndesis.

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ABSTRACTS

TORSTEN WICKBOM: The chromosomes of *Pipa pipa.*

In the author's previous works (WICKBOM, 1945, 1949 a, 1950) attempts have been made to prove how far the distribution of cytologic features really reflected the taxonomy of Anura. In these investigations he has shown that cytologically Anura are very uniform but variations occur. These variations are found exclusively in the primitive suborders Amphicoela and Opistocoela. This fact and the distribution of these cytologic features among other Vertebrates compelled the author to the opinion that they must be regarded as relics of conditions in remote ancestors (WICKBOM, 1949 a, 1950). Most important of these relics is the occurrence of acrocentric chromosomes and high chromosome numbers in *Ascaphus*, *Xenopus*, *Alytes*, and *Discoglossus*. It was shown (WICKBOM, *op. cit.*) that the evolution of the Anuran karyotypes has proceeded in two phases. Firstly, the old Eutetrapod set of 24 large and 24 small acrocentric chromosomes was transformed to 12 large and 12 small metacentrics by means of centric fusions. Secondly, these chromosomes might be changed by translocations, inversions, *etc.*

The author has made many efforts to extend these investigations on primitive Anura also to the South American Pipids, expecting that their cytology might throw some more light upon the evolution of the Anuran karyotypes.

Pipa pipa LINNÉ was described as early as 1758 and will be found in almost every handbook of zoology as an instance of brood care in Anura. The females of the genus *Pipa* carry their eggs and young in temporary pits in the dorsal skin.

According to the old taxonomy the genus *Pipa* was included, together with the African clawed toads *Xenopus*, in the suborder Aglossa while all other Anura formed the suborder Phaneroglossa. More recently, however, NOBLE (1931) divided Anura into five suborders. According to NOBLE the genera *Pipa*, *Protopipa*, *Xenopus*, *Hymenochirus*, and *Pseudhymenochirus* form the family Pipidae which together with Discoglossidae constitute the suborder Opistocoela.

Still more recently the South American Pipids were divided into three genera, *Hemipipa*, *Pipa*, and *Protopipa*, but DUNN (1948) has shown that this division is ungrounded. According to DUNN all American Pipids should be brought together in the genus *Pipa*. The characters which unite the *Pipae* with the African Pipids as well as those unique for the genus *Pipa* are analysed and tabulated by DUNN who arrives at the conclusion that *Pipa* and the African genera mentioned are closely related but that the American genus must be considered much less primitive than the African ones.

Thus, all modern taxonomists agree as to the close relationship between the American and the African Pipids.

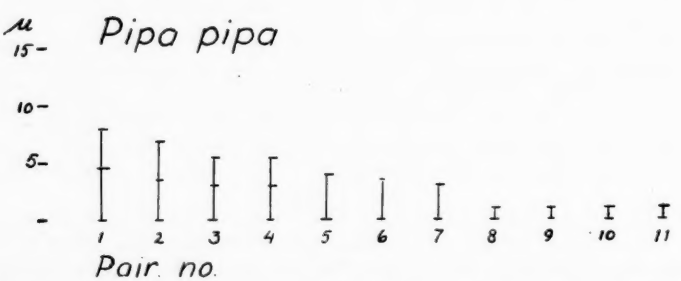
Material. — Through the great kindness of Dr. STAHEL, Paramaribo, the author has succeeded in getting material of *Pipa pipa*. The animals were caught in the surroundings of Paramaribo, Suriname, in April, 1950. The gonads were removed, fixed in acetocarmine and sent in that fluid to Sweden by air. This took four days. Immediately after arrival the testes were squashed according

to the author's method (WICKBOM, 1945). As the staining was very faint the slides were put into acetic orcein in 50° C for two hours after removal of the cover glasses. It is a pleasant duty for the author to express here his most cordial thanks to Dr. STAHEL and his collaborators for their very valuable help with this unique material which gave excellent cytologic preparations.

Results. — The testes of *Pipa pipa* were in a very active spermatogenesis. All mitotic and meiotic stages were found and analysed.

Pipa pipa has $2n = 22$ chromosomes (Fig. 1, Scheme 1). Four pairs are

Fig. 1. Mitotic metaphase of *Pipa pipa*. 2200×. — Fig. 2. I-M of *Pipa pipa*. 2200×. — Fig. 3. Diplotene of *Pipa pipa*. 2200×.



Scheme 1. Lengths of chromosomes at mitotic metaphase. Only one member of every pair is drawn. *Pipa pipa.*

large and metacentric, three large and acrocentric, and four very small and acrocentric.

At meiosis 11 bivalents are formed (Figs. 2 and 3). All bivalents have one chiasma per arm pair whence the frequency of chiasmata must be considered very low. The chiasmata are distally localized as in other Anura (WICKBOM, 1945, 1949 a). At diakinesis a major spiral is formed.

No chromosome pair can be identified as sex chromosomes by its appearance or behaviour. This is also in accordance with the conditions in other Anura (WICKBOM, 1945, 1949 a, 1950).

Discussion. — In spite of its close relationship with *Xenopus* there is not very much in the cytology of *Pipa* which, at first sight, is reminiscent of that species. In fact, the chromosome number of *Pipa* is the lowest found in Anurans. *Bufo* and *Acris* also have only 22 chromosomes (WICKBOM, 1949 b) but all these are metacentric whence the N. F. (nombre fondamental; MATTHEY, 1949) in these species is 44. The N. F. of *Pipa* is only 30.

The author has shown (WICKBOM, 1949 a, 1950) that one is justified in assuming an ancient stem set of Eutetrapoda consisting of 24 macro- and 24 microchromosomes, all acrocentric. In *Pipa* we find 14 macros, 8 meta- and 6 acrocentric; that is 22 macro arms. Evidently 2 old macrochromosomes must have disappeared. Of the 24 microchromosomes only 8 are left, the others having been lost during evolution.

As pointed out above, the closest relative of *Pipa* is *Xenopus*. In this species we find 8 metacentric and 4 acrocentric macros, 8 metacentric and 16 acrocentric micros (WICKBOM, 1949 a). *Xenopus* has possibly retained all its chromosome arms though four macros are transformed to micros. In *Pipa*, however, not only two macros but also 16 micros are lost, or two macros are transformed to micros and 18 micros lost.

Thus, there are really similarities between *Pipa* and *Xenopus*. The two species may have separated from a common stock with 8 metacentric macros at the most. Perhaps the transformation of macros to micros had begun already in this ancestor of Pipidae, but naturally the similarity on this point may be due to parallel evolution. In *Xenopus* the micros are retained, but in *Pipa* they are, to a considerable extent, lost. This is a very unique condition in Anura. Where the N. F. is lowered in other species [*Bufo*, *Acris* (WICKBOM, 1949 b)] by loss of chromosomes, this loss does not occur before all acrocentrics have fused to metacentrics.

Thus we find in *Pipa pipa* that primitive as well as very advanced cytologic features occur together. The occurrence of acrocentrics must be regarded as primitive. On the other hand, the reduction in number of chromosome arms, the existence of a maior spiral, and the low number of chiasmata are signs of a cytologic specialization. In particular, the low frequency of chiasmata suggests, to a certain extent, evolutionary stagnation.

As shown above, *Pipa pipa* belongs to the primitive suborder Opistocoela but is a very specialized species of a very specialized genus. Evidently the taxonomic position of *Pipa pipa* reflects its cytologic characteristics very well.

A cytologic investigation of some of the more primitive *Pipa* species, *e. g. Pipa parva* from Venezuela or *Pipa carvalhoi* from Pernambuco, Brazil, would be of great interest and might reveal phases in the cytologic evolution of *Pipa* which are put forward as mere hypotheses here. Material of these species, however, is extremely difficult to obtain.

Summary. — (1) The chromosome number of *Pipa pipa* is $2n = 22$, $n = 11$. — (2) Four chromosome pairs are large and metacentric, three large and acrocentric, the remaining four very small and acrocentric. — (3) The number of chiasmata is one per arm pair. — (4) A maior spiral is formed during diakinesis. — (5) No chromosome pair can be identified as the sex chromosomes. — (6) The karyotype of *Pipa pipa* is supposed to have evolved

from the hypothetical stem set of Anura by fusion of 16 macrochromosomes to 8 metacentric macros and loss of two macrochromosomes. All micros except 8 are also lost. — (7) The systematic position of *Pipa pipa* reflects the mixture of primitive and advanced features in the cytology.

*

The present investigation and the translation of this paper into English were aided by a grant from the Swedish Natural Science Research Council.

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NILS OLOF BOSEMARK: Accessory chromosomes in *Festuca pratensis* HUDS.

In 1947 the author determined the chromosome number of 78 clones of *Festuca pratensis* at Svalöf. In addition to the normal fourteen chromosomes eight clones had accessory chromosomes, *viz.* 3 clones $2n = 14 + 1$ acc., 4 clones $2n = 14 + 2$ acc. and 1 clone $2n = 14 + 4$ acc. In order to get a more representative material wild plants from all Sweden were collected in 1948 and 1949. The distribution of the chromosome numbers in this material is seen in Table 1.

TABLE 1. *Number of accessory chromosomes in plants collected in 1948—49.*

Number of acc. chr.	0	1	2	3	4	5	6	7
Number of plants	855	97	68	8	11	3	3	2

Hence a total of 1047 plants, 18,3 % of which had accessory chromosomes. The collected plants were taken quite at random with the only exception that cultivated areas were avoided.

The accessory chromosomes in my material are of the same kind as those shown by RANCKEN (1934). They do not seem to have any morphological effect, are about one-fourth of the normal chromosomes in length, have a median centromere and look like iso-chromosomes at mitosis. Studies of the pachytene stage, however, have proved that they are unsymmetrical. At this stage they have a rather strong resemblance to the »standard fragment» in rye (LIMA-DE-FARIA, 1948; MÜNTZING and LIMA-DE-FARIA, 1949).

In five plants new types of accessory chromosomes have been found. They are all smaller than the original and have probably arisen through deletions. Both at mitotic and meiotic prophase the accessory chromosomes in *Festuca pratensis* show positive heteropycnosis. In resting nuclei, too, they are generally seen as dark dots.

The type of non-disjunction at the first division in the pollen grain that has been described for the accessory chromosomes in rye (MÜNTZING, 1946) and *Anthoxanthum* (ÖSTERGREN, 1947) has been found to be at work in *Festuca pratensis* as well. The accessory chromosome lags between the anaphase groups, and both daughter chromosomes generally get included in the generative nucleus. Table 2 shows the results of four crosses made in the clone material of 1947.

TABLE 2. *Number of accessory chromosomes in F_1 of Festuca pratensis clones with different numbers of accessory chromosomes.*

Parents			F_1							
Clones		Number of acc. chr.	Number of acc. chr.							Average number of acc. chr. per plant
♀	♂	♀ ♂	0	1	2	3	4	5	6	
a 9	a10	2 × 2	1	22	9	37	—	1	—	2,2
a10	a 9	2 × 2								
12	a10	2 × 2	1	12	11	35	1	—	1	2,5
a10	12	2 × 2								
a 8	a 9	1 × 2	1	1	12	15	—	—	—	2,4
a 9	a 8	2 × 1	—	14	12	—	—	—	—	1,5
a 8	a 6	1 × 4	—	3	3	2	4	3	—	3,1
a 6	a 8	4 × 1	—	4	9	7	1	1	—	2,4

It is seen from the table that the non-disjunction gives rise to a progeny with an average number of accessory chromosomes higher than would be the case without a mechanism for increase. From these and other crosses it may also be concluded that the non-disjunction, as in *Anthoxanthum* (ÖSTERGREN, 1947) and in contrast to the case in rye (HÅKANSSON, 1948), is limited to the male side.

The pairing of the accessory chromosomes at pachytene seems to be complete, and no higher configurations than bivalents have been observed. In diakinesis the sticky properties of the accessory chromosomes rather often cause formation of multivalents, which, however, break up when metaphase is reached. At this stage the percentage of bivalents in plants with two accessory chromosomes varies between 60—95. Lagging univalents generally divide at the first division and are obviously distributed at random at the second division. The percentage of eliminated accessory chromosomes seen as micronuclei in the tetrads is very low. The acccessory chromosomes have not been seen to disturb the pairing of the normal chromosomes, nor has any true pairing between accessory chromosomes and normal ones been observed.

A vitality trial laid out in the spring of 1949, when harvested in the autumn, did not show any correlation between plant vitality and number of

accessory chromosomes. The tested plants, however, probably had too low numbers of accessory chromosomes, and therefore no conclusions can yet be drawn in this respect. Still, the distribution of the numbers of accessory chromosomes in the collected material makes it very probable that a limiting factor is at work in wild populations. A more detailed report will appear later.

*

Acknowledgement. — I wish to thank The Swedish Seed Association for kind permission to use their clone material of *Festuca pratensis.*

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NILS FRIES: Selective isolation of guanine-deficient mutants in *Ophiostoma.*

Most of the purine-deficient mutants isolated in various microorganisms respond to hypoxanthine or adenine. In the Ascomycete, *Ophiostoma multi-annulatum*, however, four mutants were found requiring guanine, and incapable of growing with the two other common purines. They had been isolated in the years 1945 and 1946 after x-ray irradiation, together with many other nutritional mutations, by using an enrichment technique based upon the differential development of normal and mutant mycelia in a minimal agar medium supplemented with a very low percentage of various amino-acids, vitamins, nucleosides, *etc.* The conidia to be plated out had first been treated according to a filtration method (FRIES, 1947) by which the majority of the unmutated conidia were screened out. This method of isolation did, of course, not permit any estimation of the frequency of the mutations to guanine-deficiency.

In the following four years several hundreds of various biochemical mutations were isolated in a great number of experiments including treatment with x-ray or ultraviolet irradiation and various chemical mutagens. Although c. 120 hypoxanthine-less and 30 pyrimidine-less mutants were obtained, no single case of guanine-deficiency appeared. In all these experiments, however, the treated conidia were plated out in a complete agar medium, containing a fairly high percentage of yeast-extract, malt-extract, and hydrolysed casein.

The possibility of a selection against certain types of mutants in such a rich medium must be considered (LEIN, MITCHELL and HOULAHAN, 1948).

In studying the physiology of the guanine-less mutants, it was found (FRIES, 1949) that they are inhibited in their development by hypoxanthine or adenine. The hypothesis was therefore advanced that the apparently very poor representation of this type of mutation in *Ophiostoma* (and probably also in other organisms studied) is due to the fact that the content of adenine in the »complete» media prevents the mutated conidia of this sort from germinating. If this is the case, the guanine-less mutants should appear in a medium without adenine (but supplemented with guanine).

In order to test this hypothesis, a few experiments were performed with a minimum agar medium containing 100 mg guanosine per liter, *i. e.* about 1 mg per plate. However, it soon proved to be necessary also to add a very small amount of malt-extract, 0,2 per cent, in order to induce the dense hyphal growth and rich conidial production which is necessary for the mass-isolation technique employed (FRIES, 1948). In this medium the amounts of adenine and hypoxanthine contained in the malt-extract were exceeded many times by the quantity of guanosine present. As I wished to have at least some idea of the frequency of the mutations to guanine-deficiency, every sort of enrichment technique was avoided. Before plating out, the conidia were treated with ultraviolet irradiation until c. 95 % were killed.

In the five experiments, 8339 monoconidial mycelia were picked up and three (0,03 %) guanine-less mutants were found among them, one in each of the last three experiments. Some other types of mutations were also obtained, as a consequence of the small amount of malt-extract added to the substrate.

Expt. No.	% surviving conidia	No. of monoconidial mycelia isolated	No. of guanine-less mutants isolated
899	2,6	1338	0
902	7,8	858	0
903	3,2	2489	1
906	7,2	846	1
908	2,2	2808	1

The results strongly support the hypothesis referred to above. It is thus obvious that, despite the comparatively low frequency with which the mutation (or mutations) to guanine-deficiency occur, at least in *Ophiostoma*, any desired number of such mutants may be obtained by repeated experiments of the type now described, where the inhibiting effect of adenine is eliminated.

Institute of Physiological Botany, University of Uppsala, June 6, 1950.

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